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(54) **CELLS, COMPOSITIONS AND METHODS**

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(73) Assignee: **Genome Research Limited**, London (GB)

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Publication Classification

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A61P 31/12	(2006.01)
A61K 35/12	(2006.01)

(52) **U.S. Cl.** **424/93.71**; 435/375; 435/325

(57) **ABSTRACT**

Method of producing induced T-to-Natural-Killer [ITNK] cells, target T cells and/or target pro-T cells from T cells and/or pro-T cells which method involves modulating the activity and/or effect of at least one Bcl11b gene and/or protein present in a T cell and/or pro-T cell, and converting said T cell and/or pro-T cell to an ITNK cell or target Tcells and/or target pro-T cells is described. ITNK cells, target T cells and/or target pro-T cells produced by such method and mature activated T cells in which Bcl11b expression is down-regulated or absent, and the use of such cells or modulators of Bcl11b in medicine is also described.

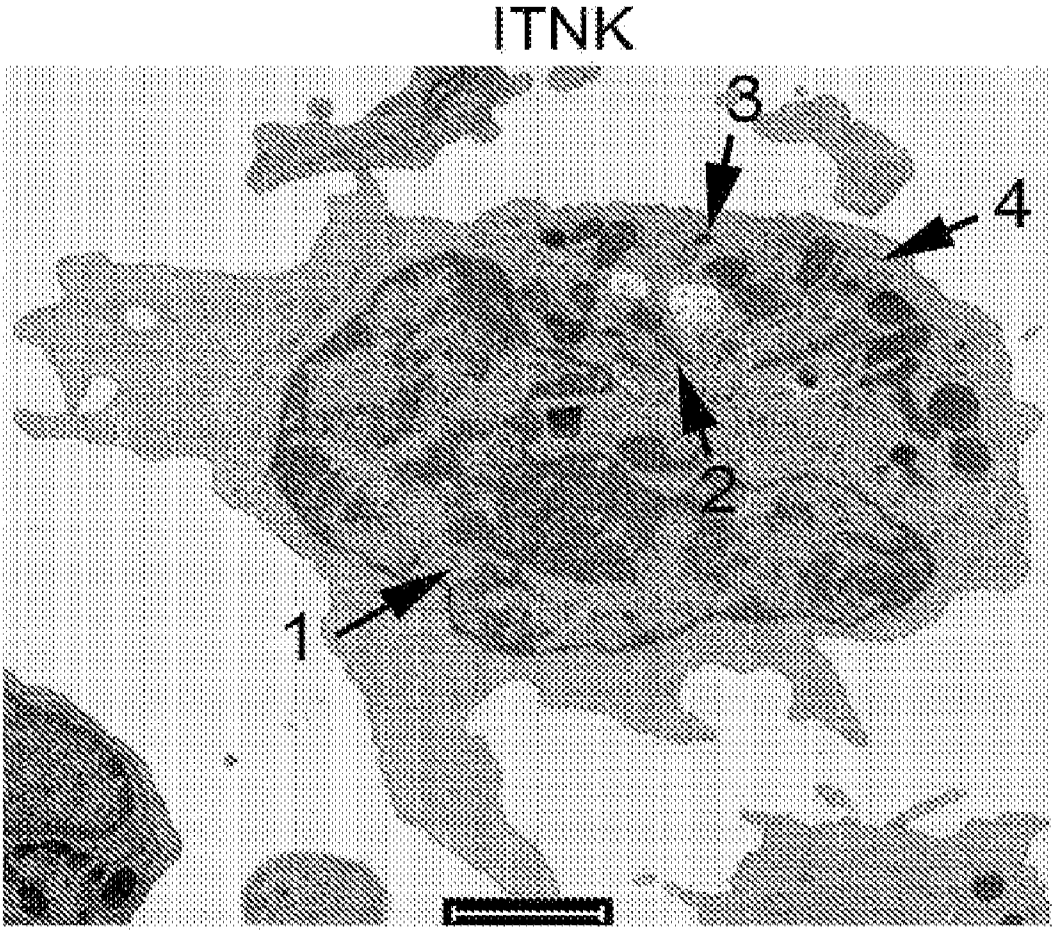
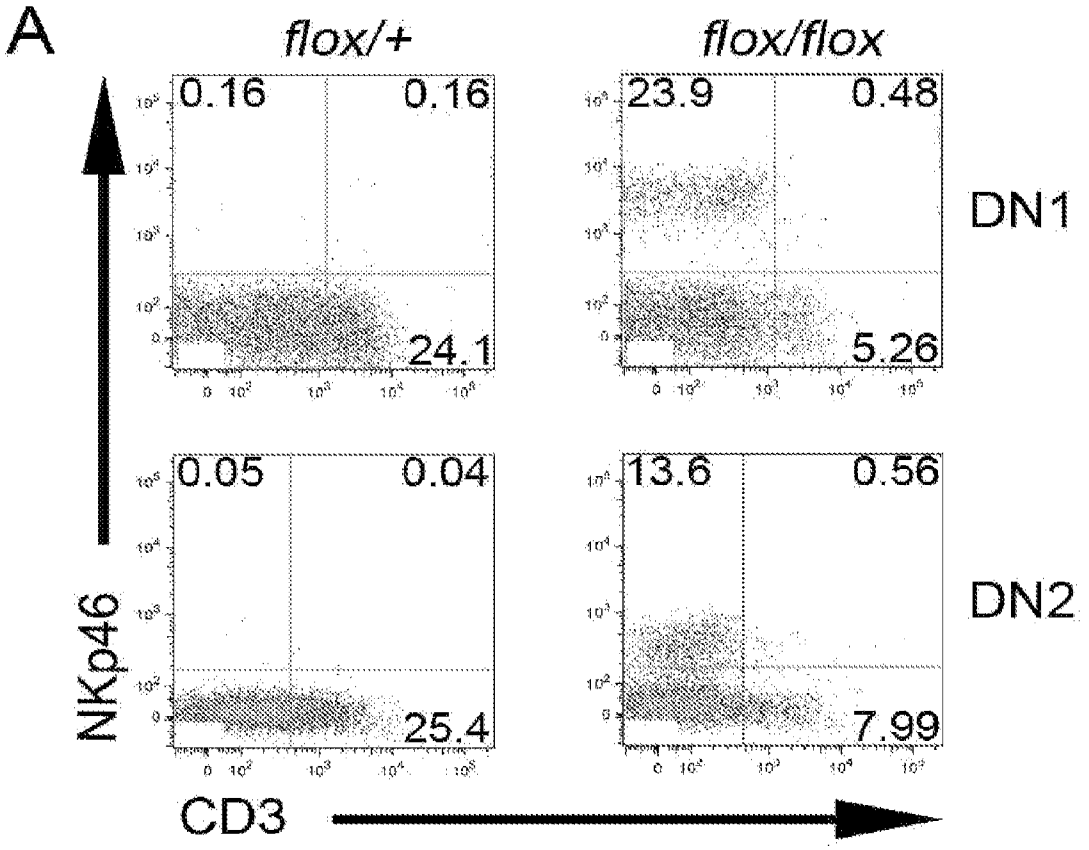


Figure 1


A
flox/+
flox/flox
0.16
0.16
24.1
23.9
0.48
5.26
DN1
0.05
0.04
25.4
13.6
0.56
7.99
DN2
NKp46
CD3

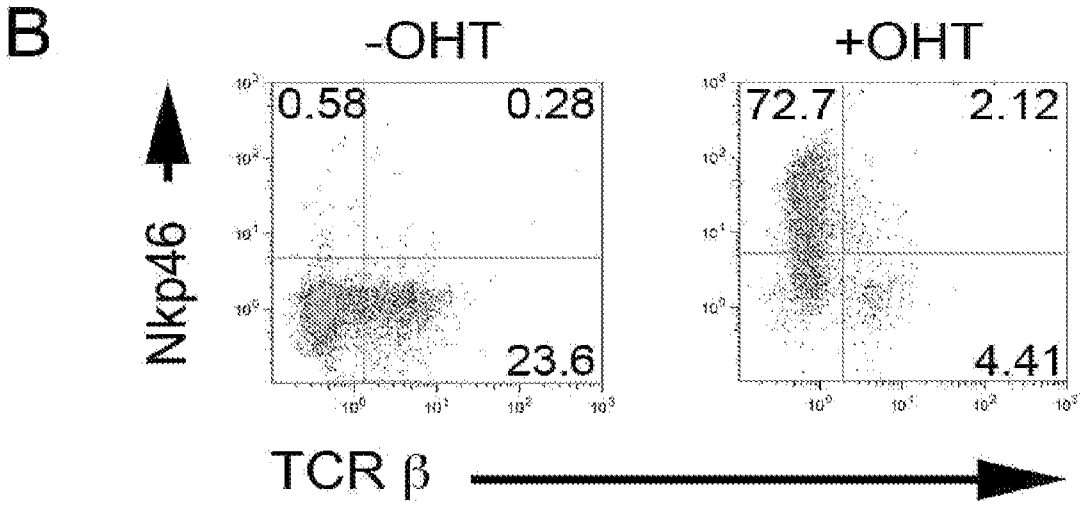

B
-OHT
+OHT
0.58
0.28
23.6
72.7
2.12
4.41
Nkp46
TCR β

Figure 1

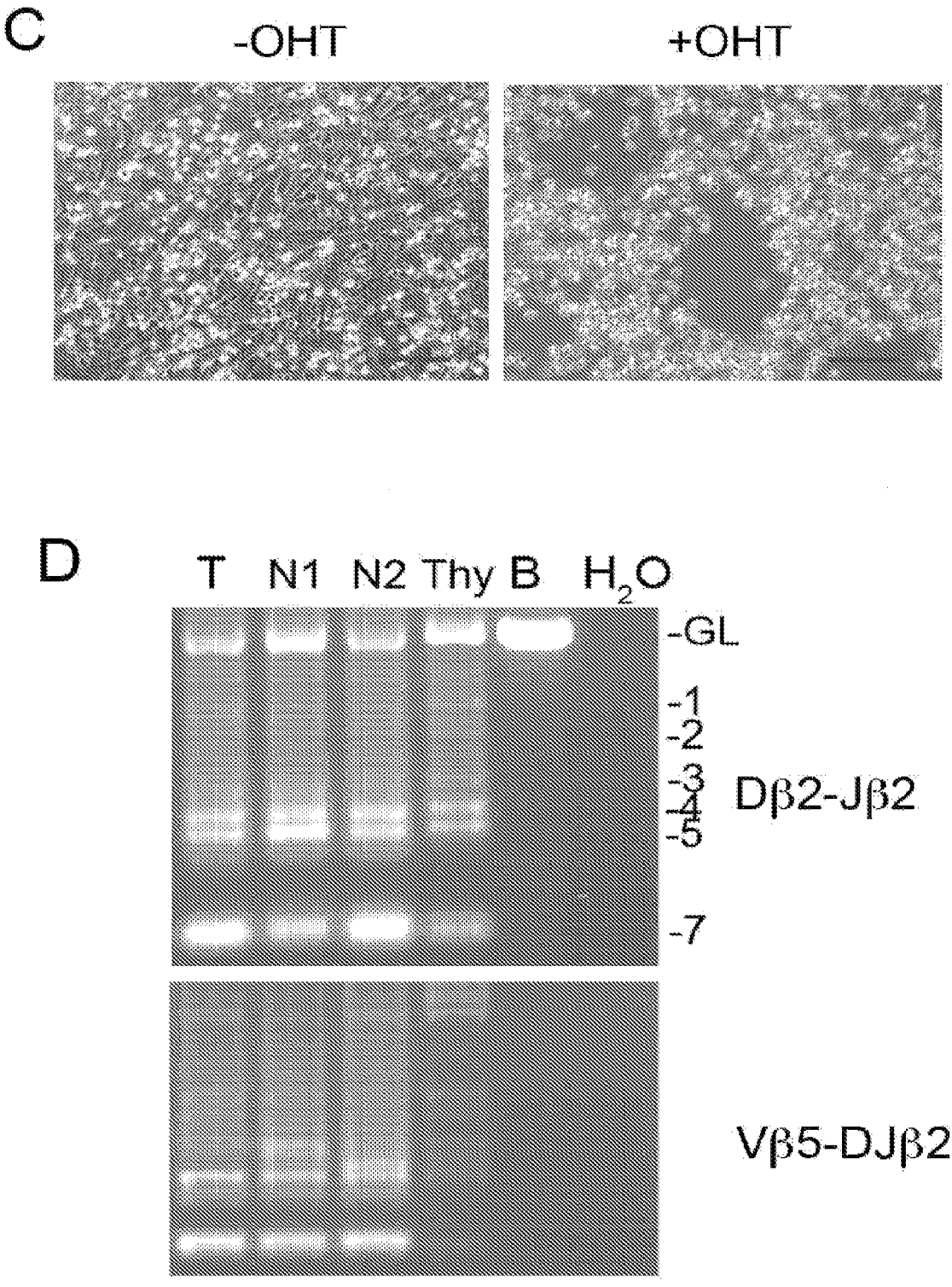


Figure 1

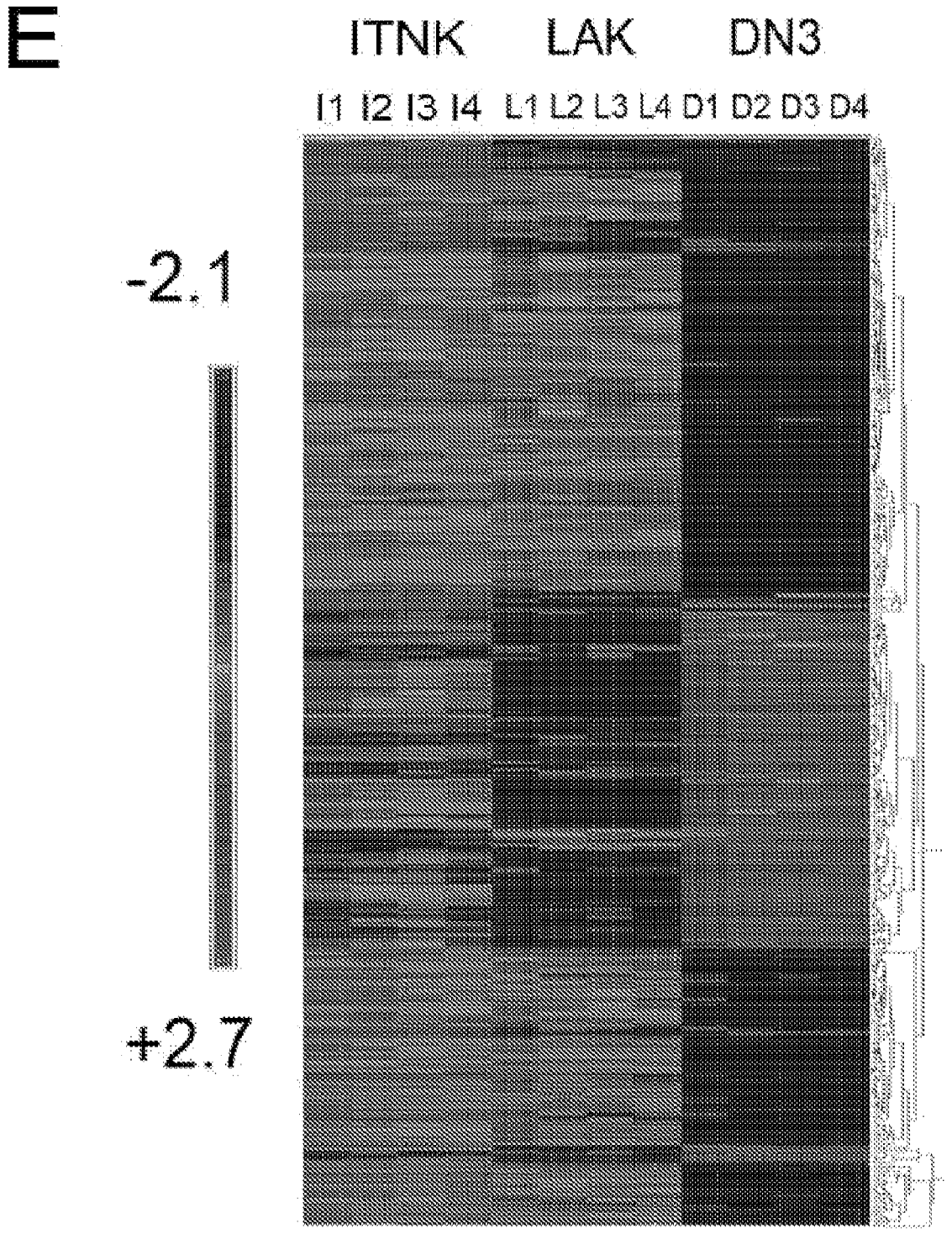
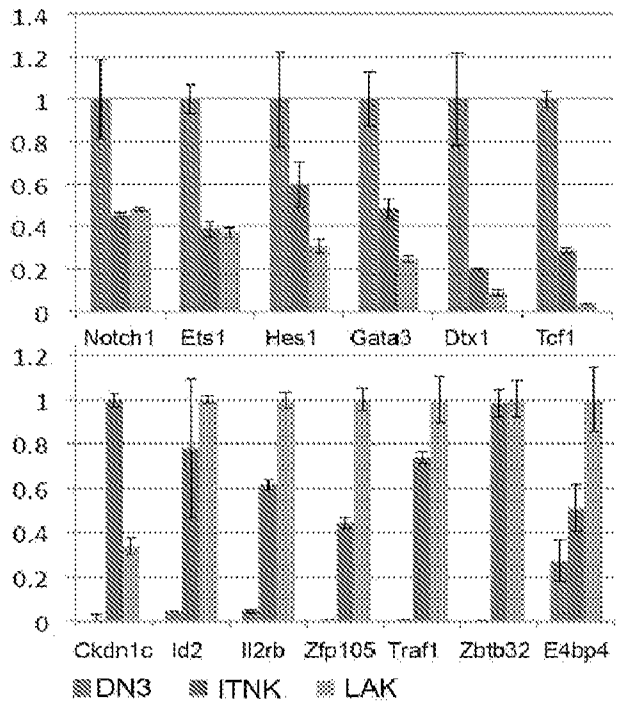


Figure 1

F


1.4
1.2
1
0.8
0.6
0.4
0.2
0
Notch1
Ets1
Hes1
Gata3
Dtx1
Tcf1
Ckdn1c
Id2
Il2rb
Zfp105
Traf1
Zbtb32
E4bp4
DN3
ITNK
LAK

G

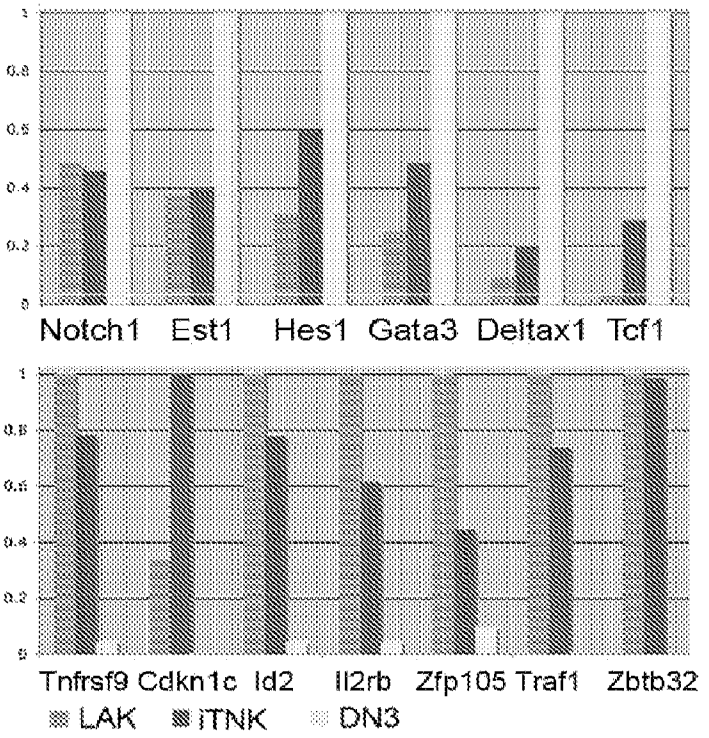
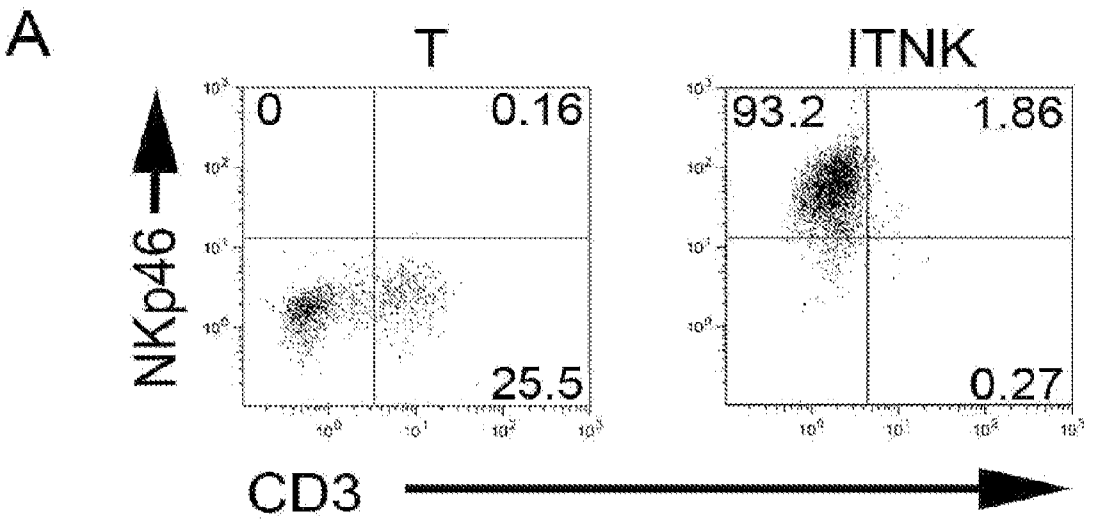

Notch1
Est1
Hes1
Gata3
Deltax1
Tcf1
Tnfrsf9
Cdkn1c
Id2
Il2rb
Zfp105
Traf1
Zbtb32
LAK
iTNK
DN3

Figure 2


A
T
ITNK
0
0.16
25.5
93.2
1.86
0.27
NKp46
CD3

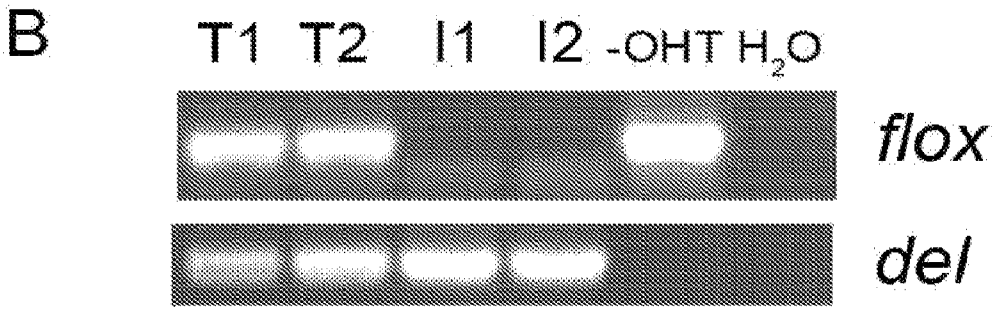
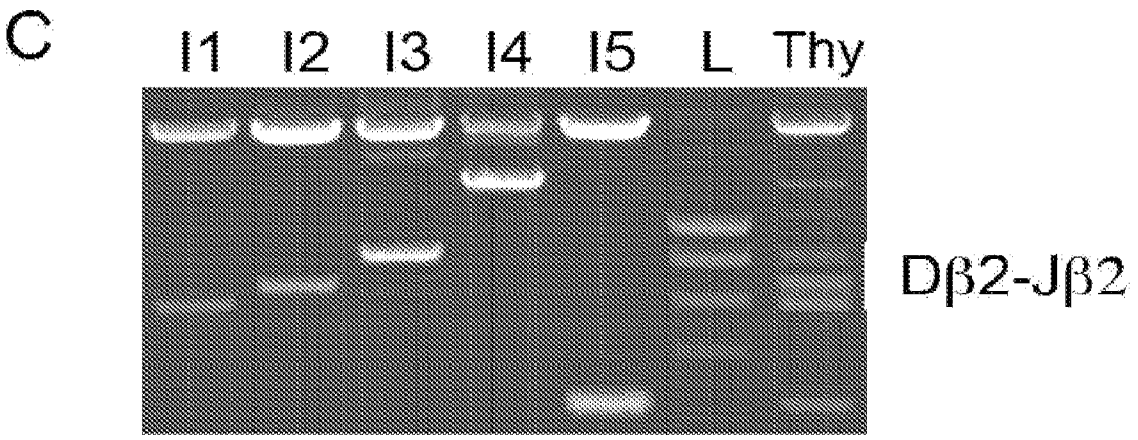

B
T1 T2 I1 I2 -OHT H2O
flox
del

Figure 2



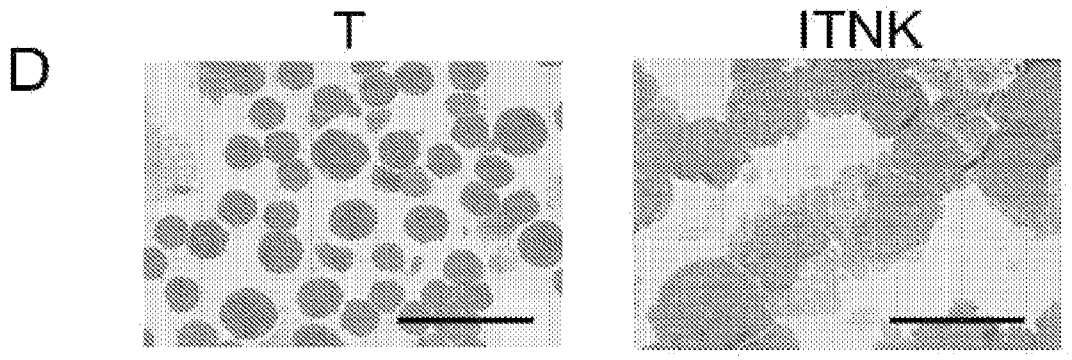
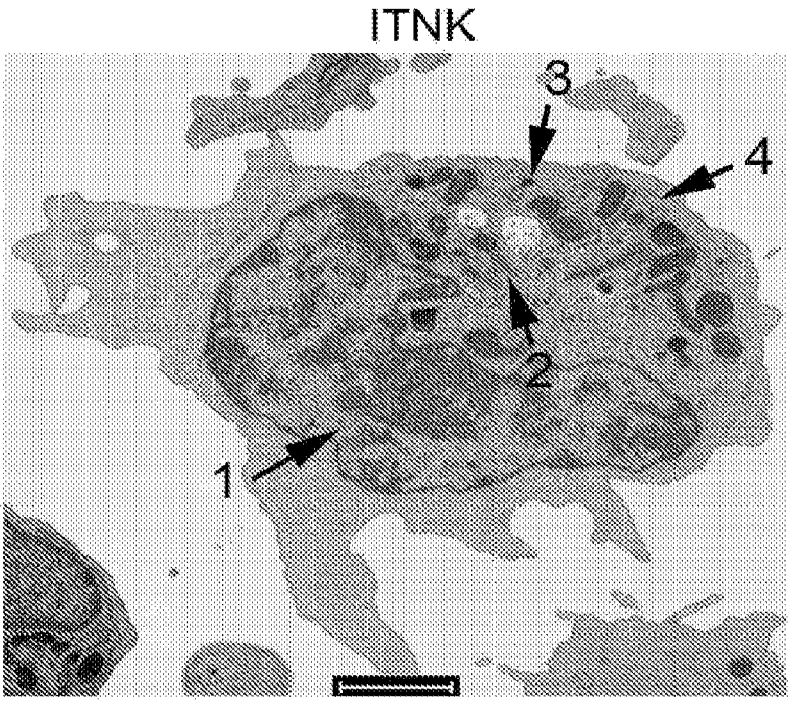


Figure 2

E



e

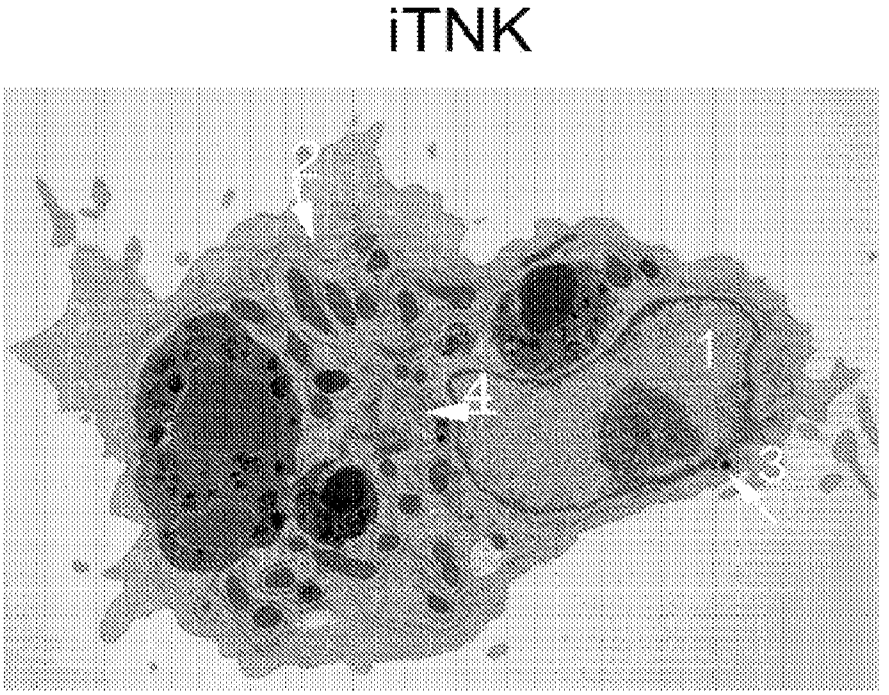


Figure 2

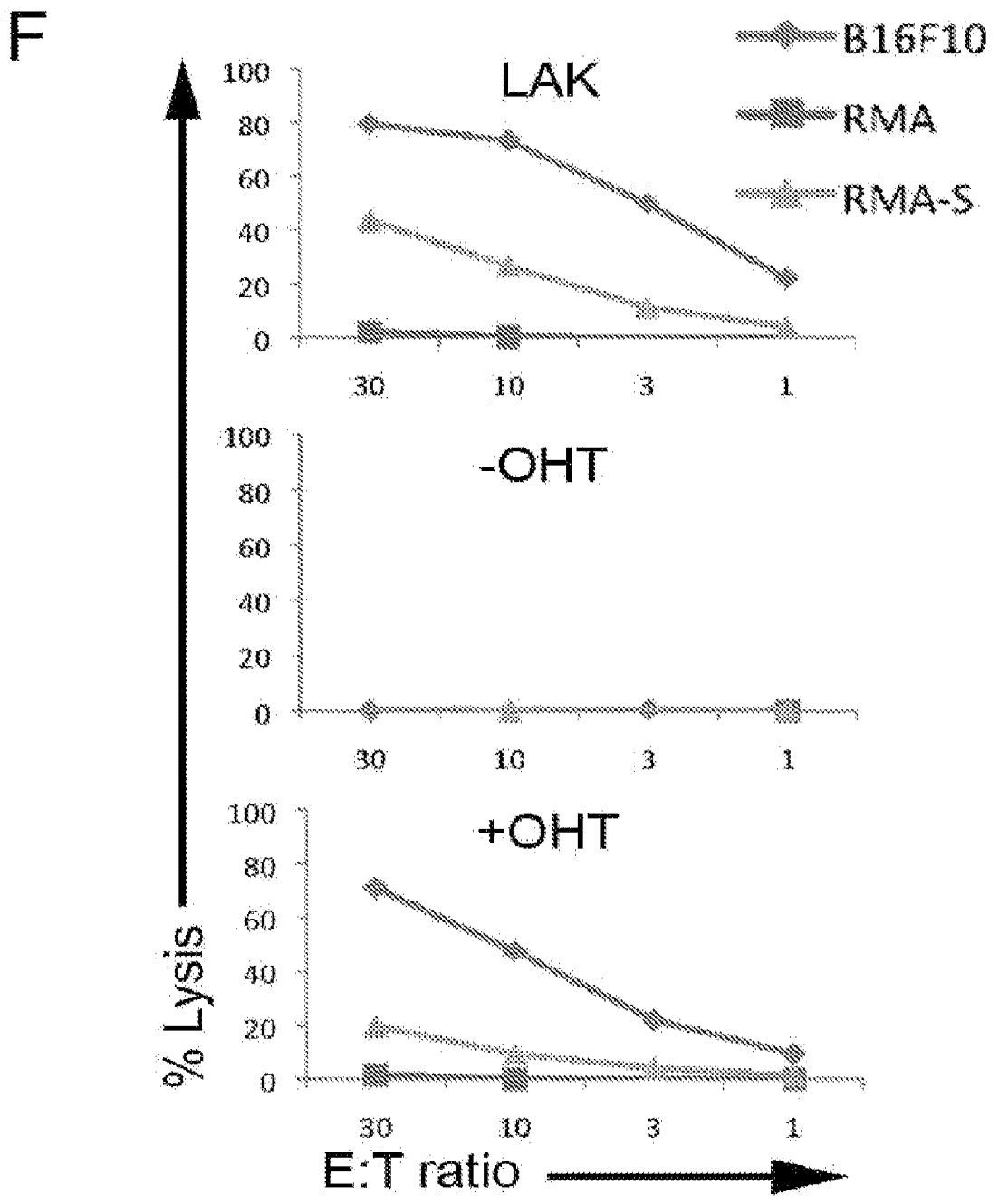

F
B16F10
RMA
RMA-S
LAK
-OHT
+OHT
% Lysis
E:T ratio
100
80
60
40
20
0
30
10
3
1

Figure 3

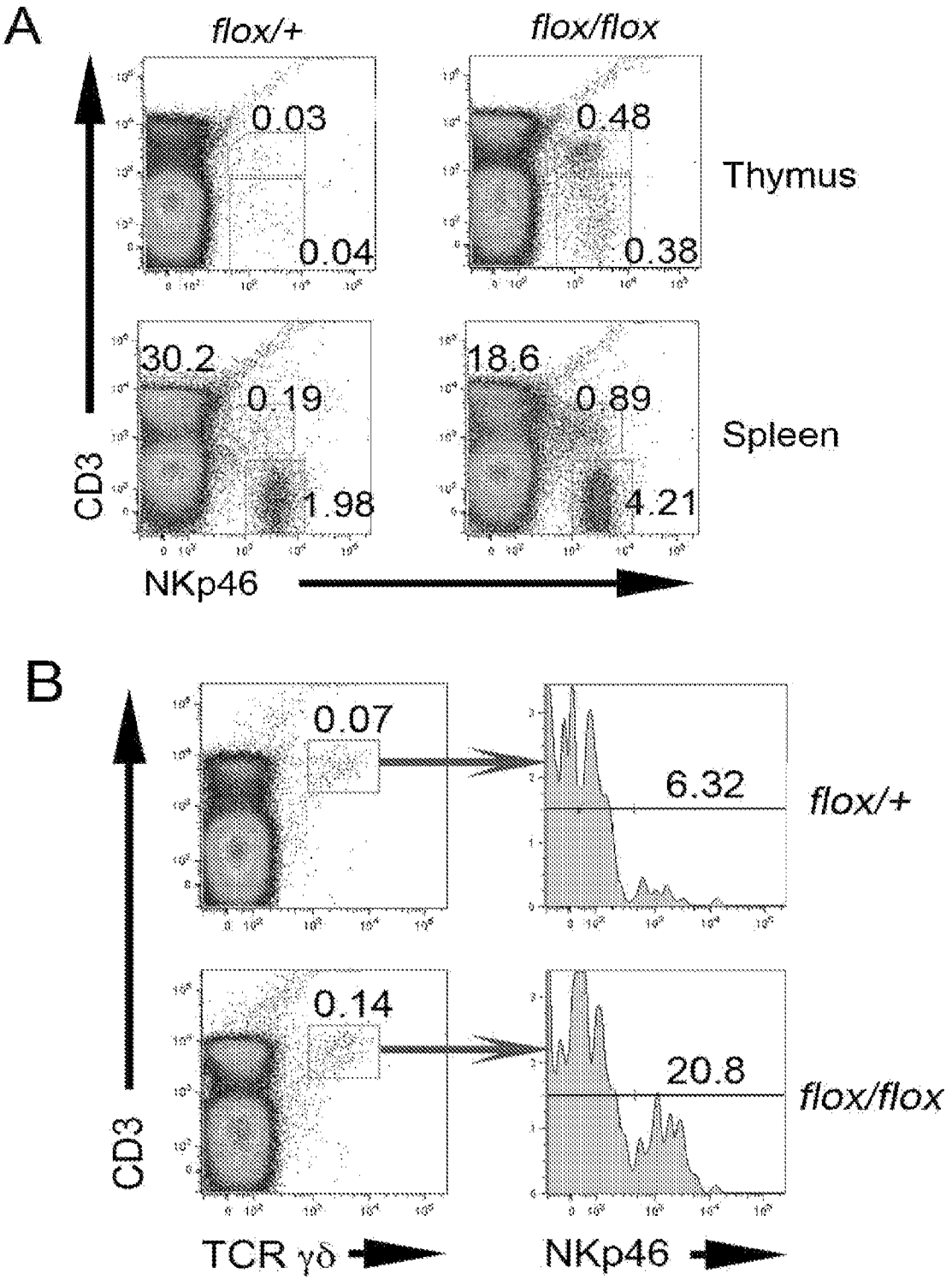

A
flox/+
flox/flox
0.03
0.04
0.48
0.38
Thymus
30.2
0.19
1.98
18.6
0.89
4.21
Spleen
CD3
NKp46
B
0.07
6.32
flox/+
0.14
20.8
flox/flox
CD3
TCR γδ
NKp46

Figure 3

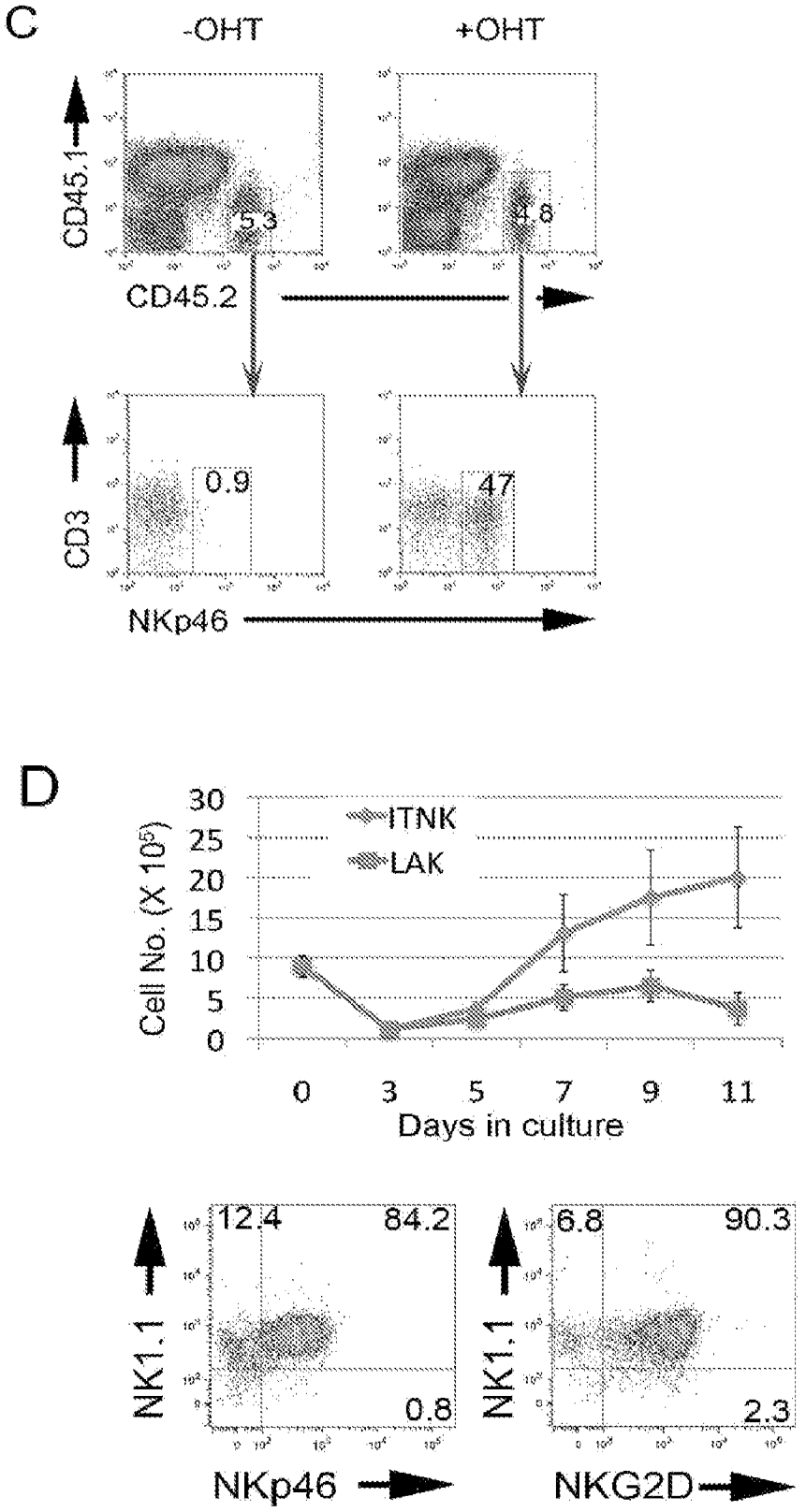
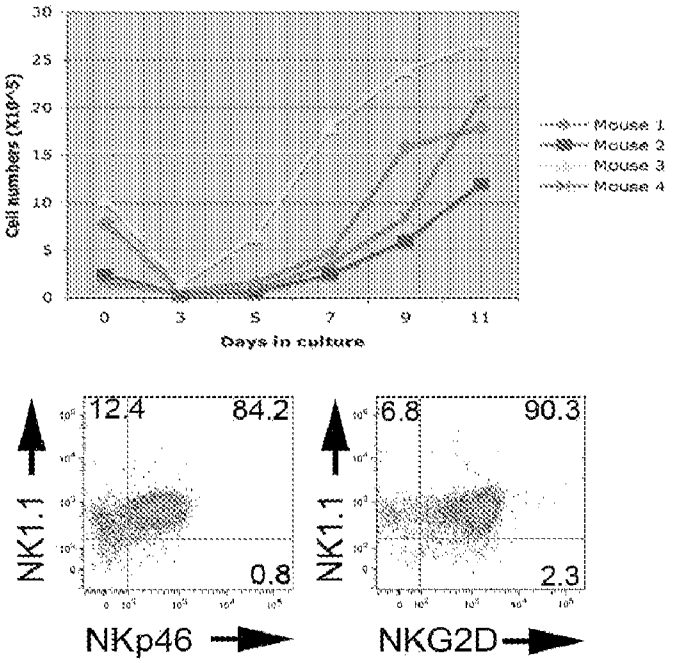

C
-OHT
+OHT
CD45.1
CD45.2
5.3
4.8
CD3
NKp46
0.9
47
D
Cell No. (X 10^5)
ITNK
LAK
Days in culture
0
3
5
7
9
11
NK1.1
NKp46
12.4
84.2
0.8
NKG2D
6.8
90.3
2.3

Figure 3 **d**


Cell numbers (X10^5)
Days in culture
Mouse 1
Mouse 2
Mouse 3
Mouse 4
12.4
84.2
0.8
NK1.1
NKp46
6.8
90.3
2.3
NK1.1
NKG2D

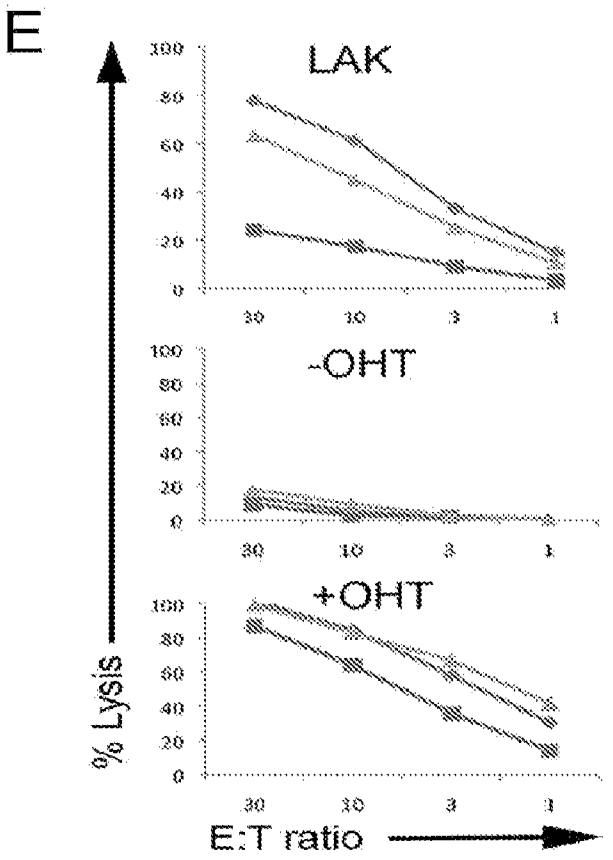
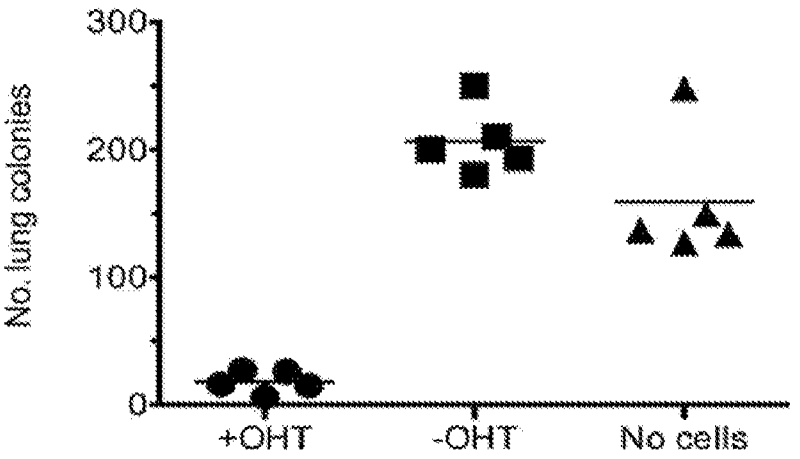

E
LAK
-OHT
+OHT
% Lysis
E:T ratio

Figure 3

F


300
200
100
0
No. lung colonies
+OHT
-OHT
No cells

G

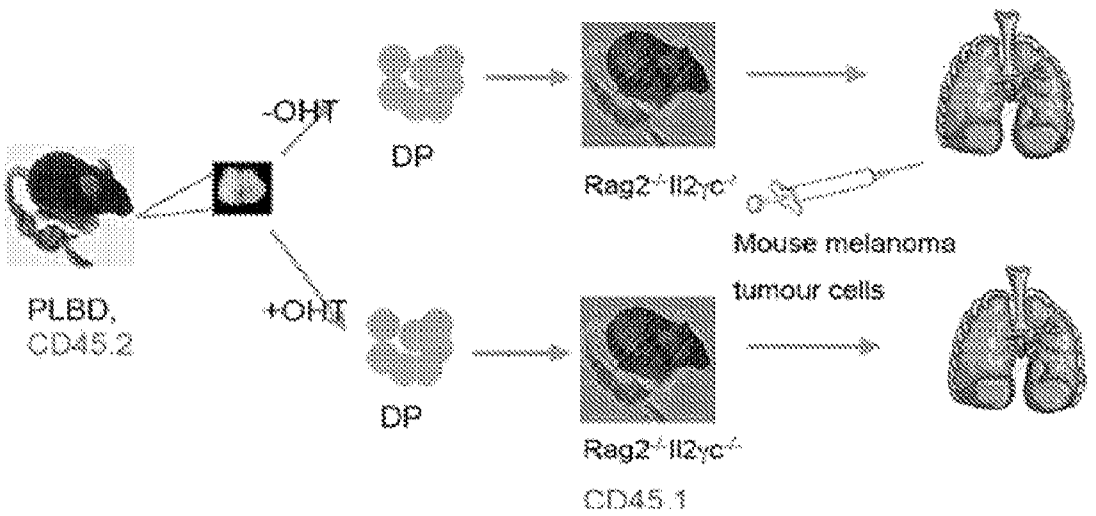

-OHT
DP
Rag2-/-Il2rγc-/-
Mouse melanoma
tumour cells
PLBD,
CD45.2
+OHT
DP
Rag2-/-Il2rγc-/-
CD45.1

Figure 4

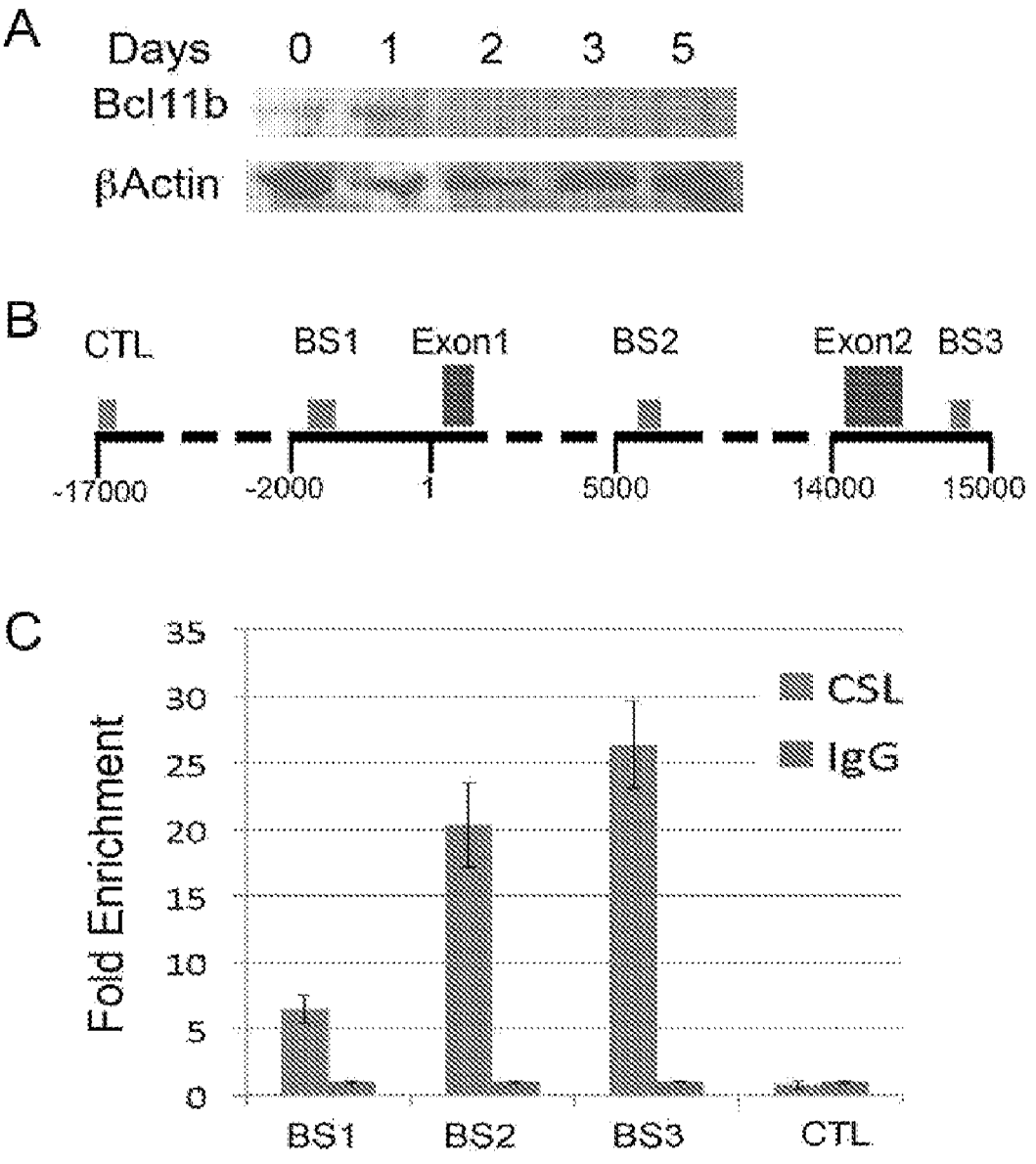

A
Days 0 1 2 3 5
Bcl11b
βActin
B
CTL
BS1
Exon1
BS2
Exon2
BS3
-17000
-2000
1
5000
14000
15000
C
Fold Enrichment
35
30
25
20
15
10
5
0
CSL
IgG
BS1
BS2
BS3
CTL

Figure 5

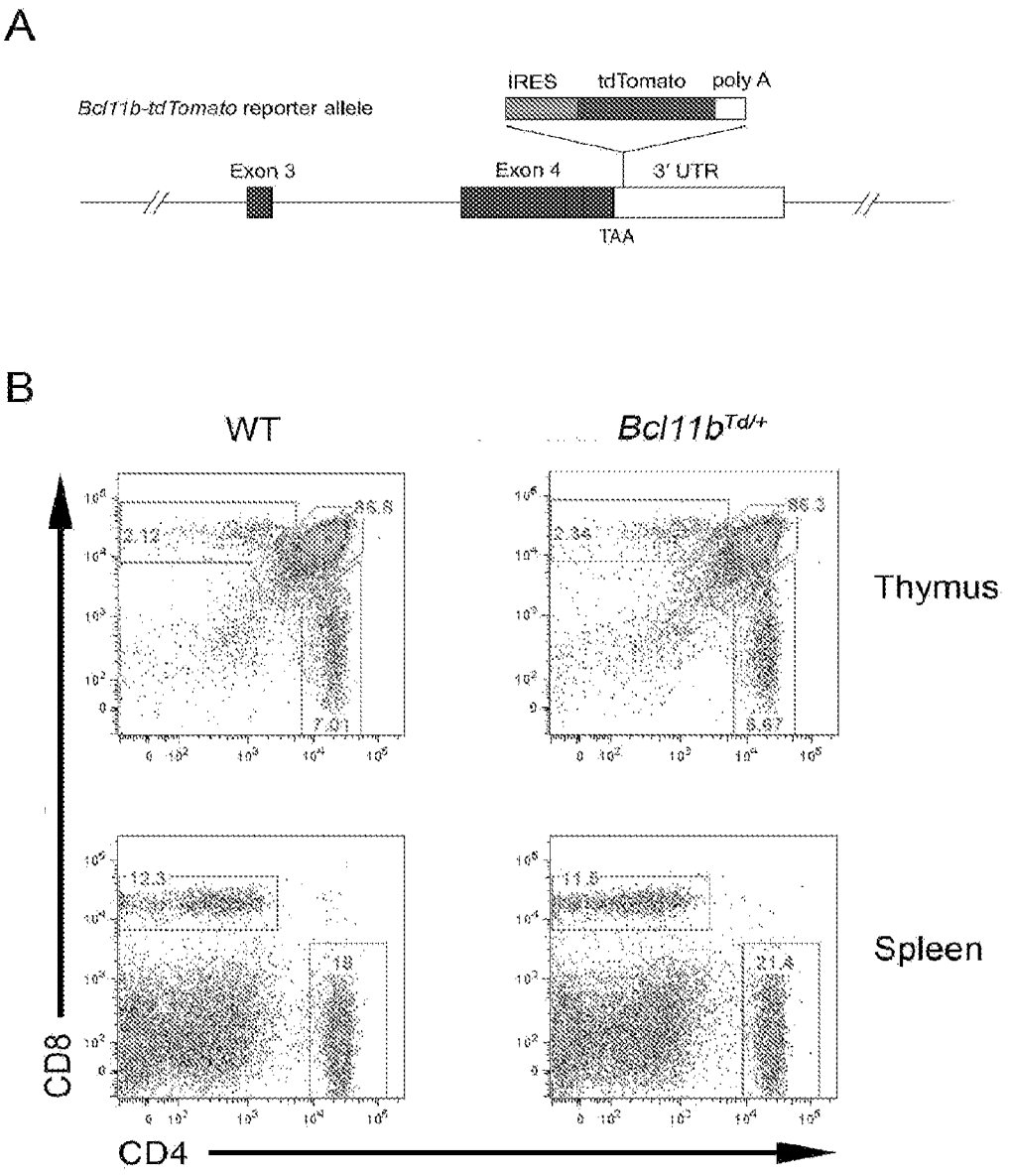

A
IRES
tdTomato
poly A
Bcl11b-tdTomato reporter allele
Exon 3
Exon 4
3' UTR
TAA
B
WT
Bcl11b^Td/+
Thymus
Spleen
CD8
CD4

Figure 6

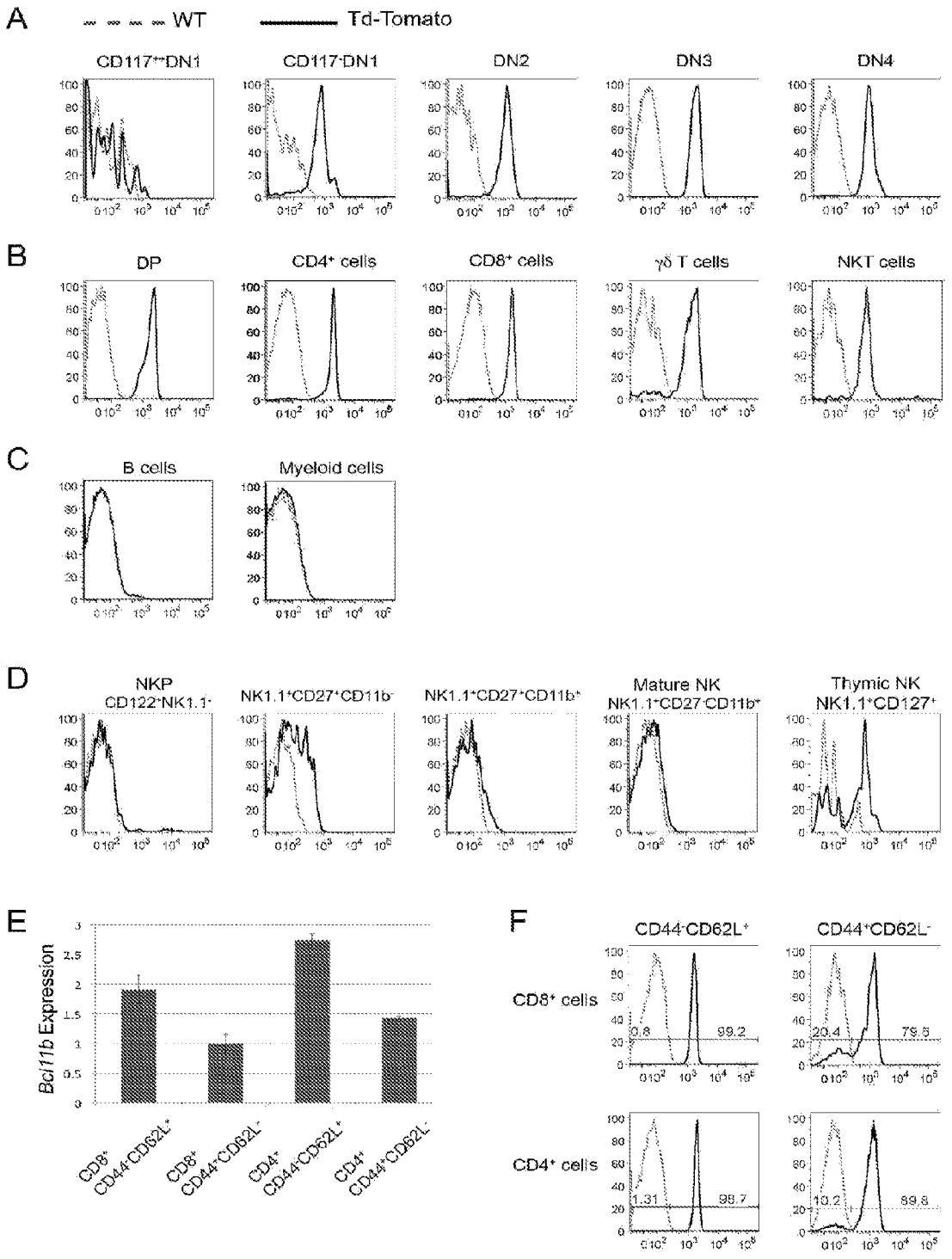

A
WT
Td-Tomato
CD117+/-DN1
CD117-DN1
DN2
DN3
DN4
B
DP
CD4+ cells
CD8+ cells
γδ T cells
NKT cells
C
B cells
Myeloid cells
D
NKP
CD122+NK1.1-
NK1.1+CD27+CD11b-
NK1.1+CD27+CD11b+
Mature NK
NK1.1+CD27-CD11b+
Thymic NK
NK1.1+CD127+
E
Bcl11b Expression
CD8+ CD44+CD62L+
CD8+ CD44+CD62L-
CD4+ CD44+CD62L+
CD4+ CD44+CD62L-
F
CD44+CD62L+
CD44+CD62L-
CD8+ cells
CD4+ cells
0.8
99.2
20.4
79.5
1.31
98.7
10.2
89.8

Figure 7

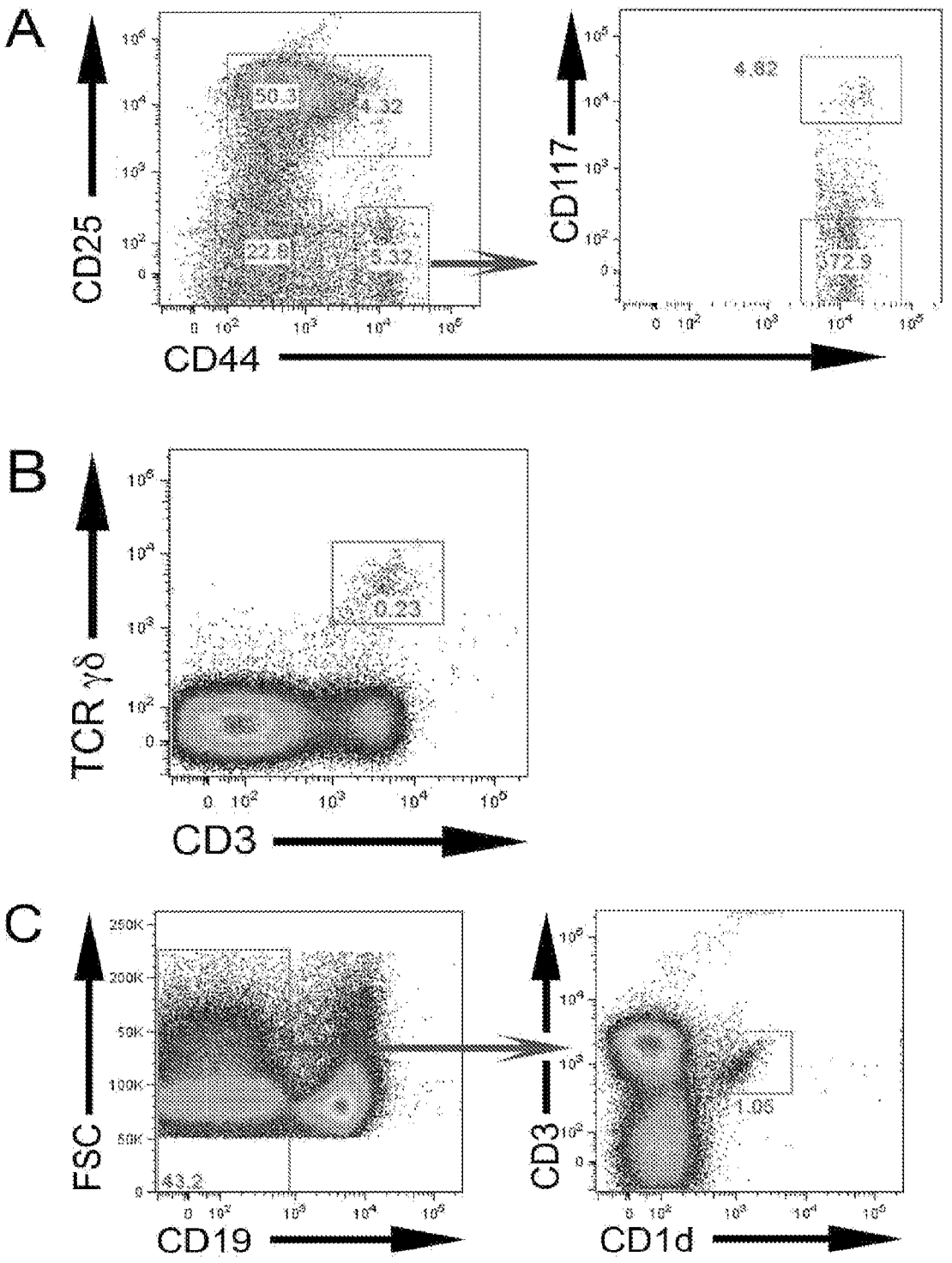

A
CD25
CD44
50.3
4.32
22.8
5.32
CD117
4.82
72.9
B
TCR γδ
CD3
0.23
C
FSC
CD19
63.2
CD3
CD1d
1.08

Figure 7

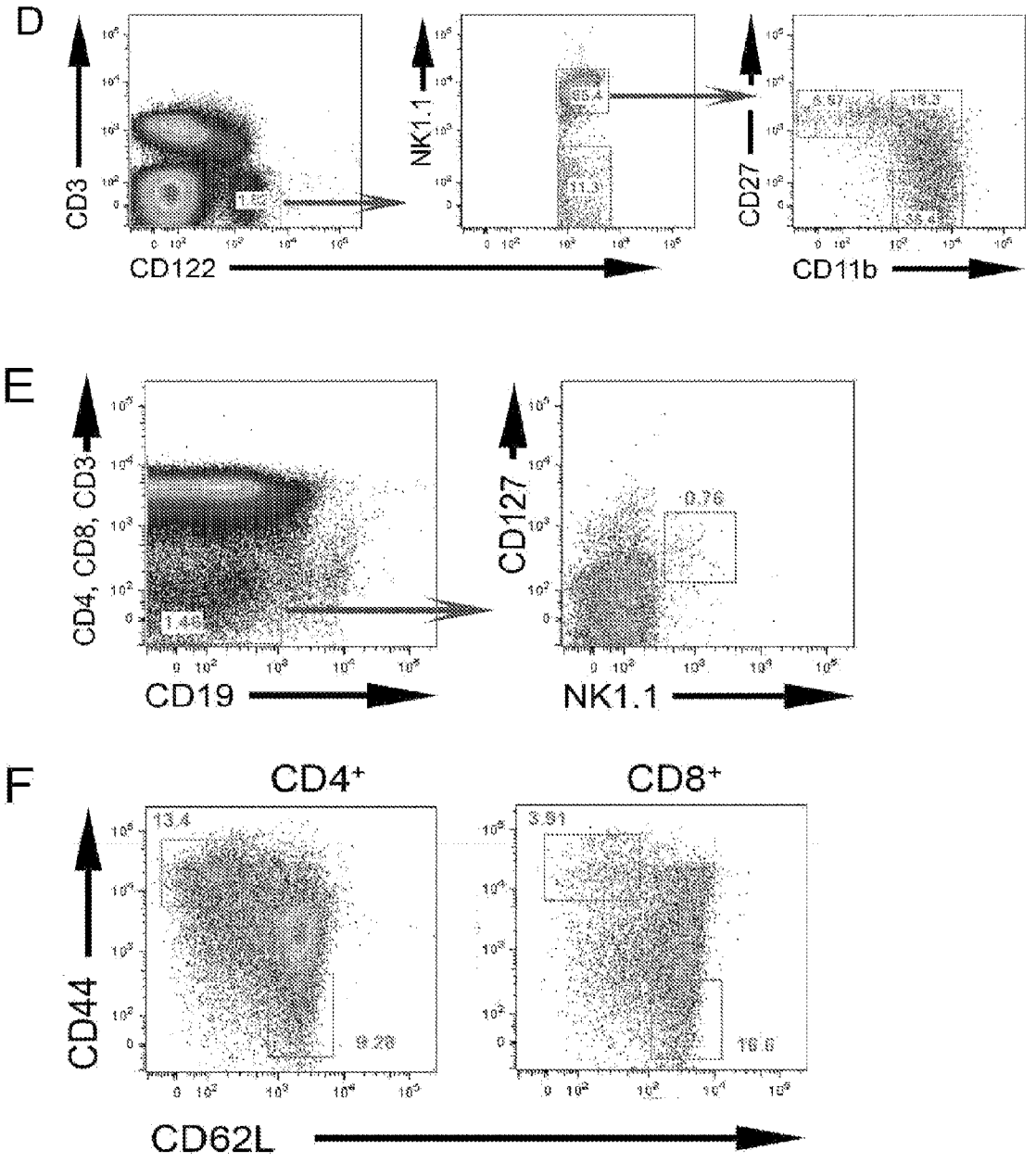
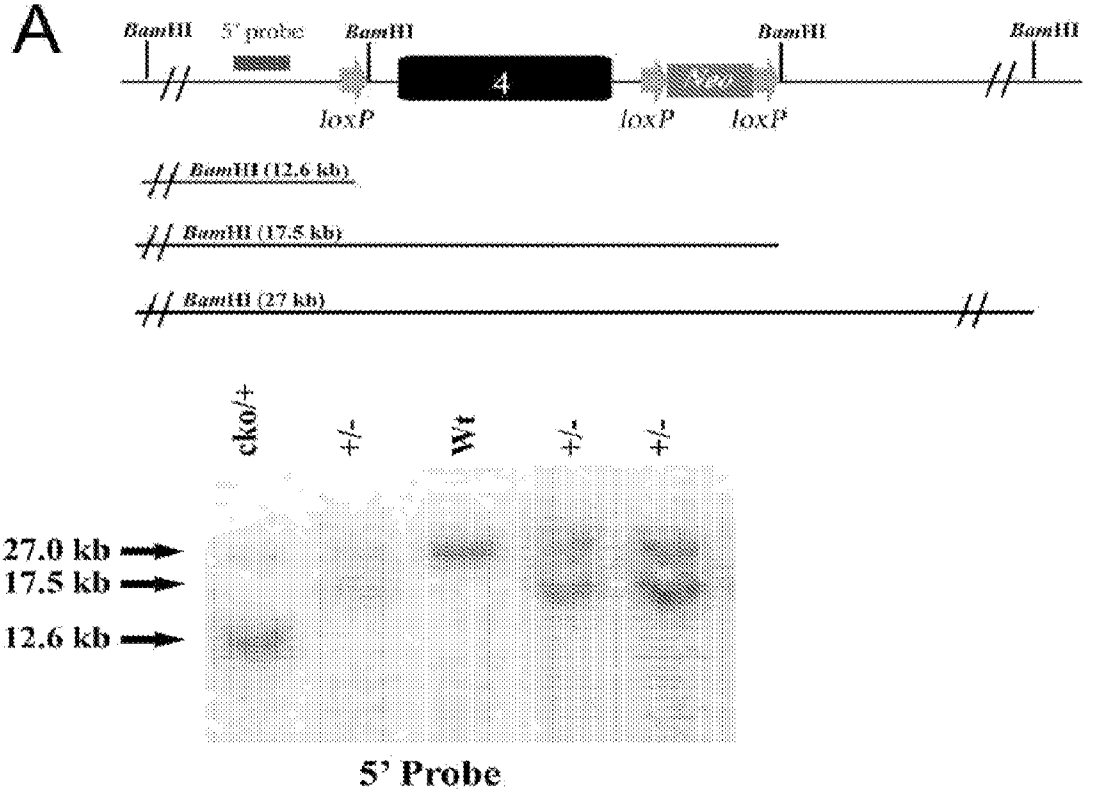

D
CD3
CD122
NK1.1
CD27
CD11b
E
CD4, CD8, CD3
CD19
CD127
NK1.1
0.76
F
CD4+
CD8+
CD44
CD62L
13.4
9.28
3.91
16.6

Figure 8


A
BamHI
5' probe
BamHI
4
BamHI
BamHI
loxP
loxP
loxP
BamHI (12.6 kb)
BamHI (17.5 kb)
BamHI (27 kb)
cko/+
+/-
Wt
+/-
+/-
27.0 kb
17.5 kb
12.6 kb
5' Probe

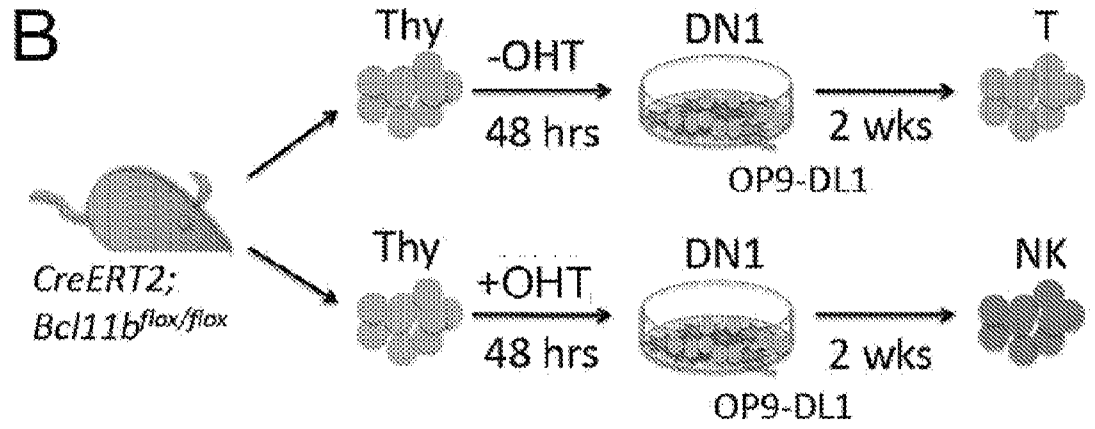
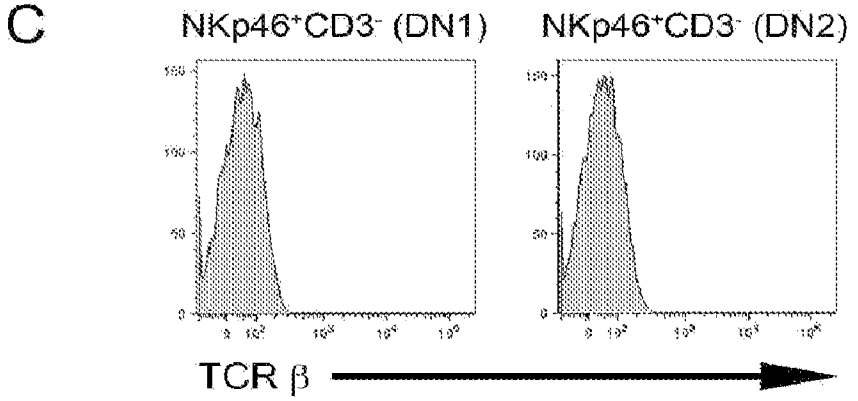
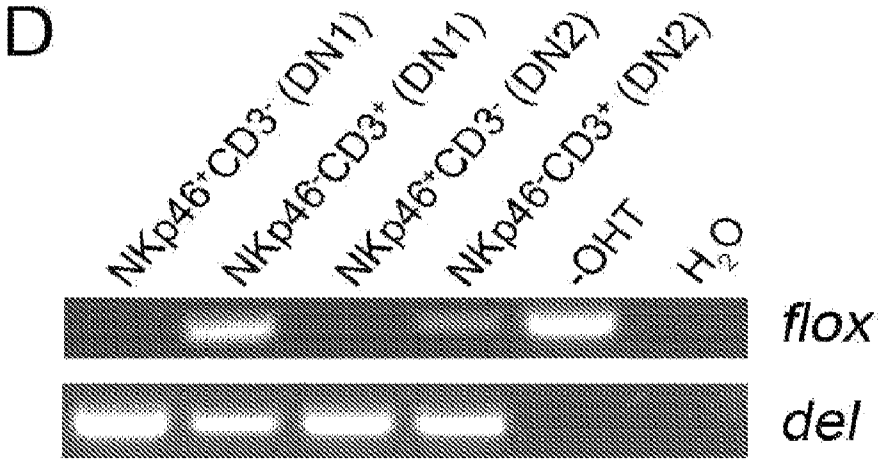

B
Thy
-OHT
48 hrs
DN1
2 wks
T
OP9-DL1
CreERT2;
Bcl11b flox/flox
Thy
+OHT
48 hrs
DN1
2 wks
NK
OP9-DL1

Figure 8


C
NKp46+CD3- (DN1)
NKp46+CD3- (DN2)
TCR β


D
NKp46+CD3- (DN1)
NKp46-CD3+ (DN1)
NKp46+CD3- (DN2)
NKp46-CD3+ (DN2)
-OHT
H2O
flox
del

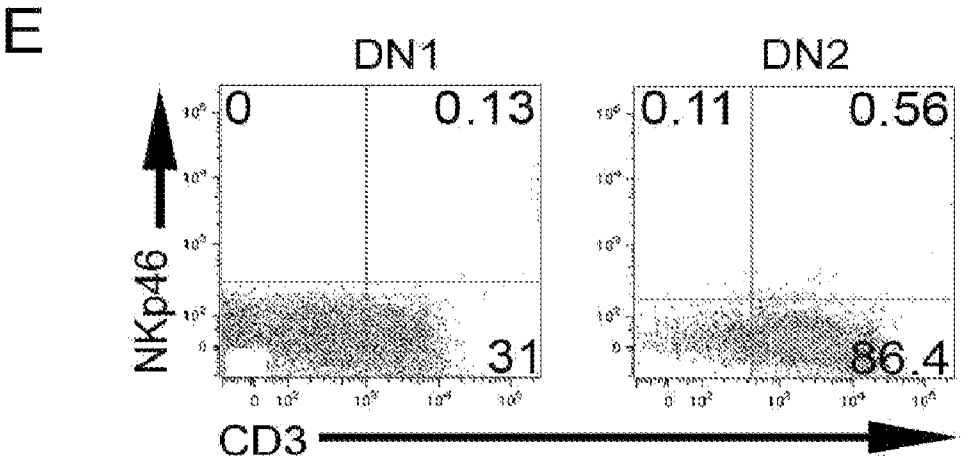
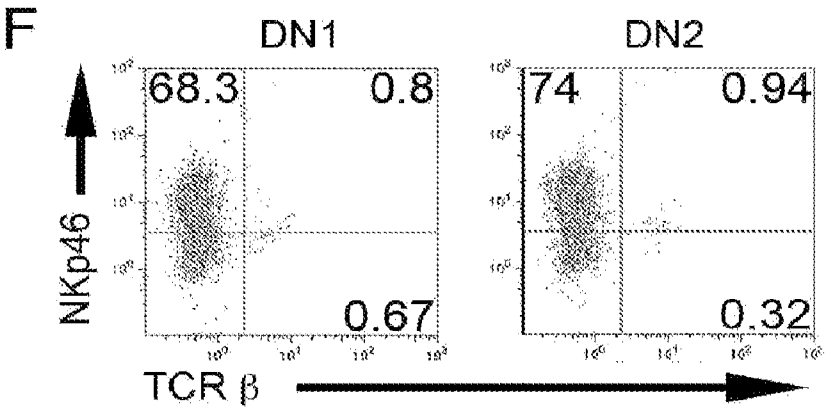
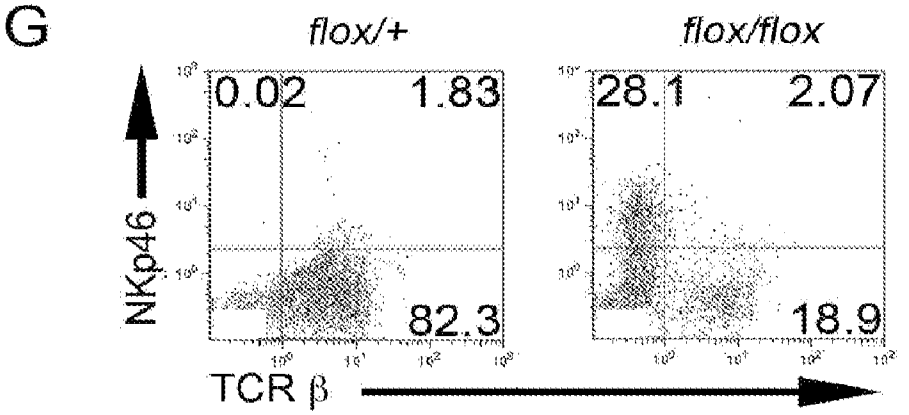

E
DN1
DN2
0
0.13
31
0.11
0.56
86.4
NKp46
CD3

Figure 8


F
DN1
DN2
68.3
0.8
0.67
74
0.94
0.32
NKp46
TCR β


G
flox/+
flox/flox
0.02
1.83
82.3
28.1
2.07
18.9
NKp46
TCR β

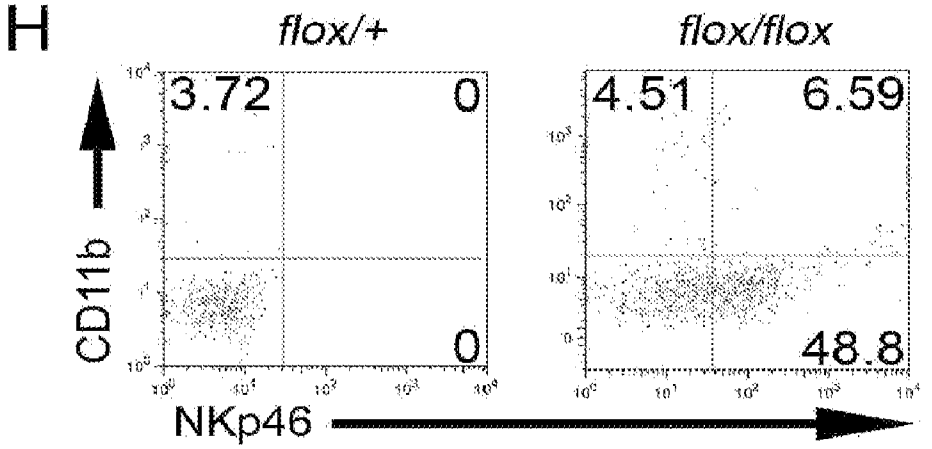
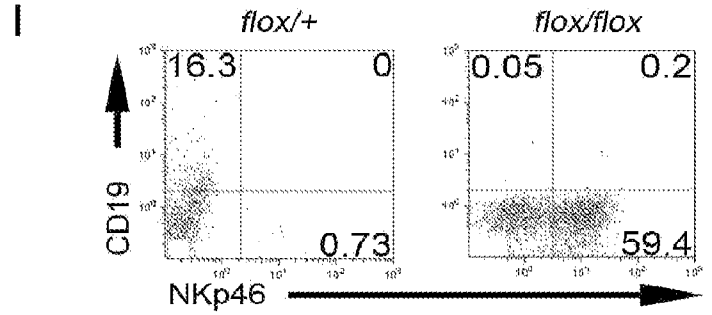
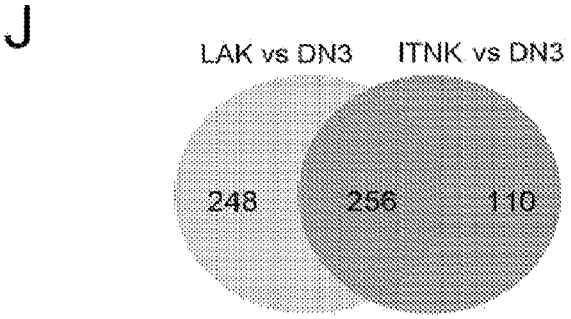
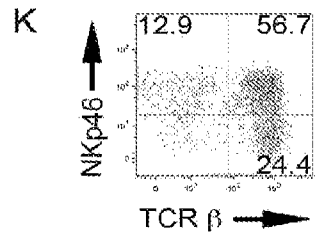

H
flox/+
flox/flox
3.72
0
0
4.51
6.59
48.8
CD11b
NKp46

Figure 8


I
flox/+
flox/flox
16.3
0
0.73
0.05
0.2
59.4
CD19
NKp46


J
LAK vs DN3
ITNK vs DN3
248
256
110


K
12.9
56.7
24.4
NKp46
TCR β

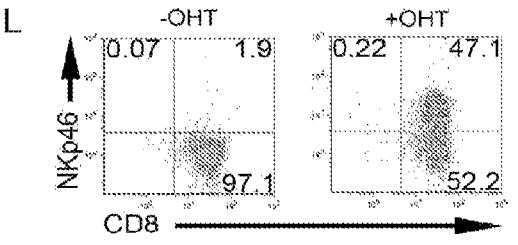
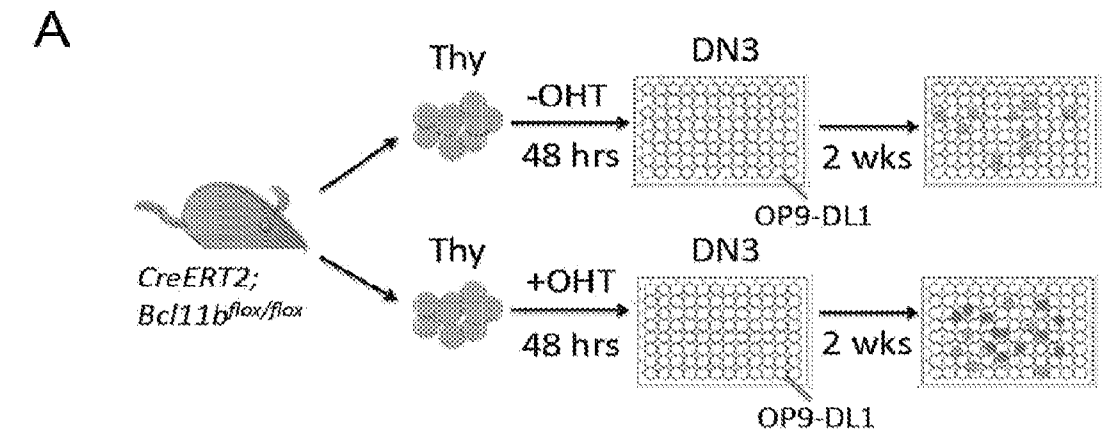

L
-OHT
+OHT
0.07
1.9
97.1
0.22
47.1
52.2
NKp46
CD8

Figure 9


A
Thy
DN3
-OHT
48 hrs
2 wks
OP9-DL1
CreERT2;
Bcl11b flox/flox
Thy
DN3
+OHT
48 hrs
2 wks
OP9-DL1

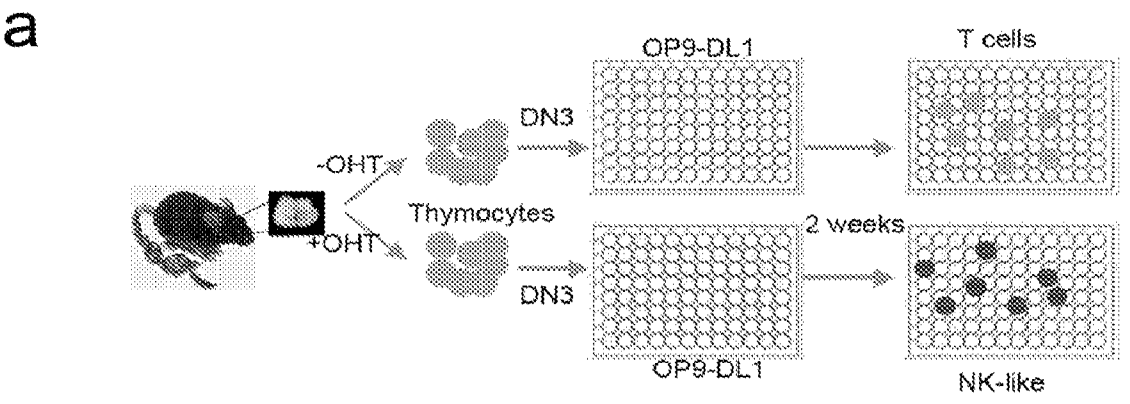

a
OP9-DL1
T cells
DN3
-OHT
Thymocytes
+OHT
2 weeks
DN3
OP9-DL1
NK-like

Figure 9

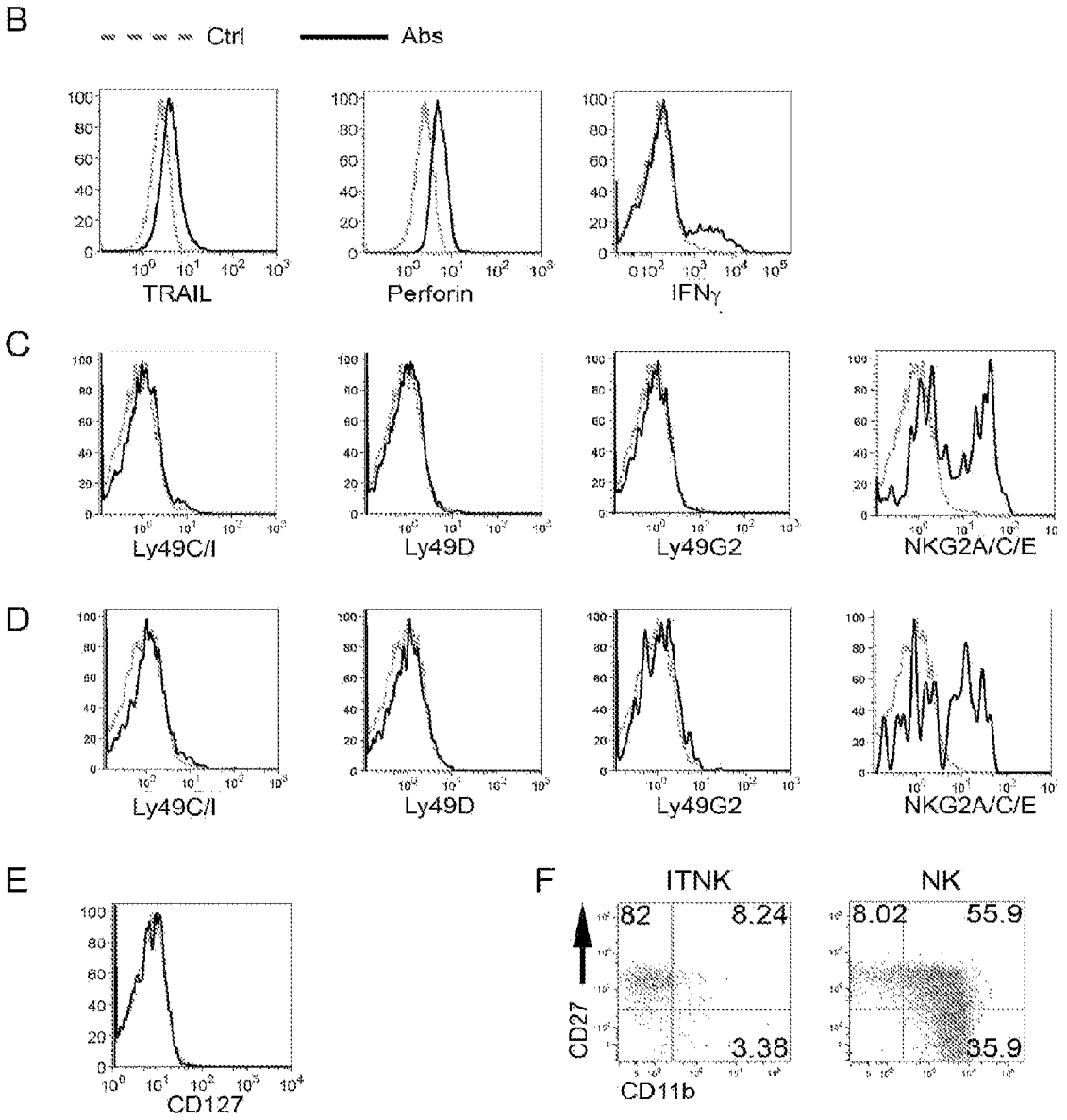

B
Ctrl
Abs
TRAIL
Perforin
IFNγ
C
Ly49C/I
Ly49D
Ly49G2
NKG2A/C/E
D
Ly49C/I
Ly49D
Ly49G2
NKG2A/C/E
E
CD127
F
ITNK
NK
82
8.24
3.38
8.02
55.9
35.9
CD27
CD11b

Figure 10

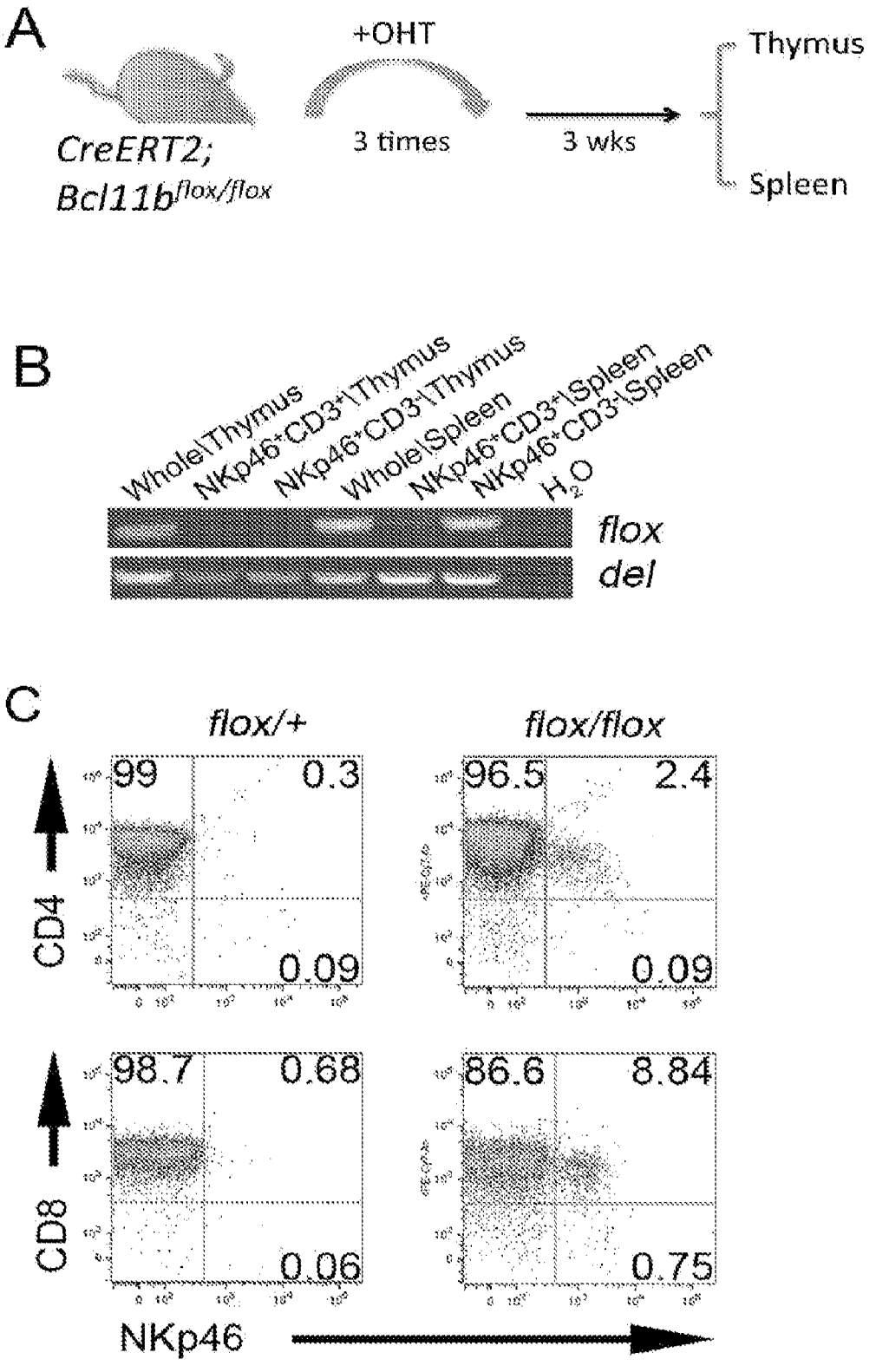

A
+OHT
3 times
3 wks
Thymus
Spleen
CreERT2;
Bcl11b flox/flox
B
Whole\Thymus
NKp46+CD3-\Thymus
NKp46+CD3-\Thymus
Whole\Spleen
NKp46+CD3+\Spleen
NKp46+CD3-\Spleen
H2O
flox
del
C
flox/+
flox/flox
99
0.3
0.09
96.5
2.4
0.09
98.7
0.68
0.06
86.6
8.84
0.75
CD4
CD8
NKp46

Figure 10

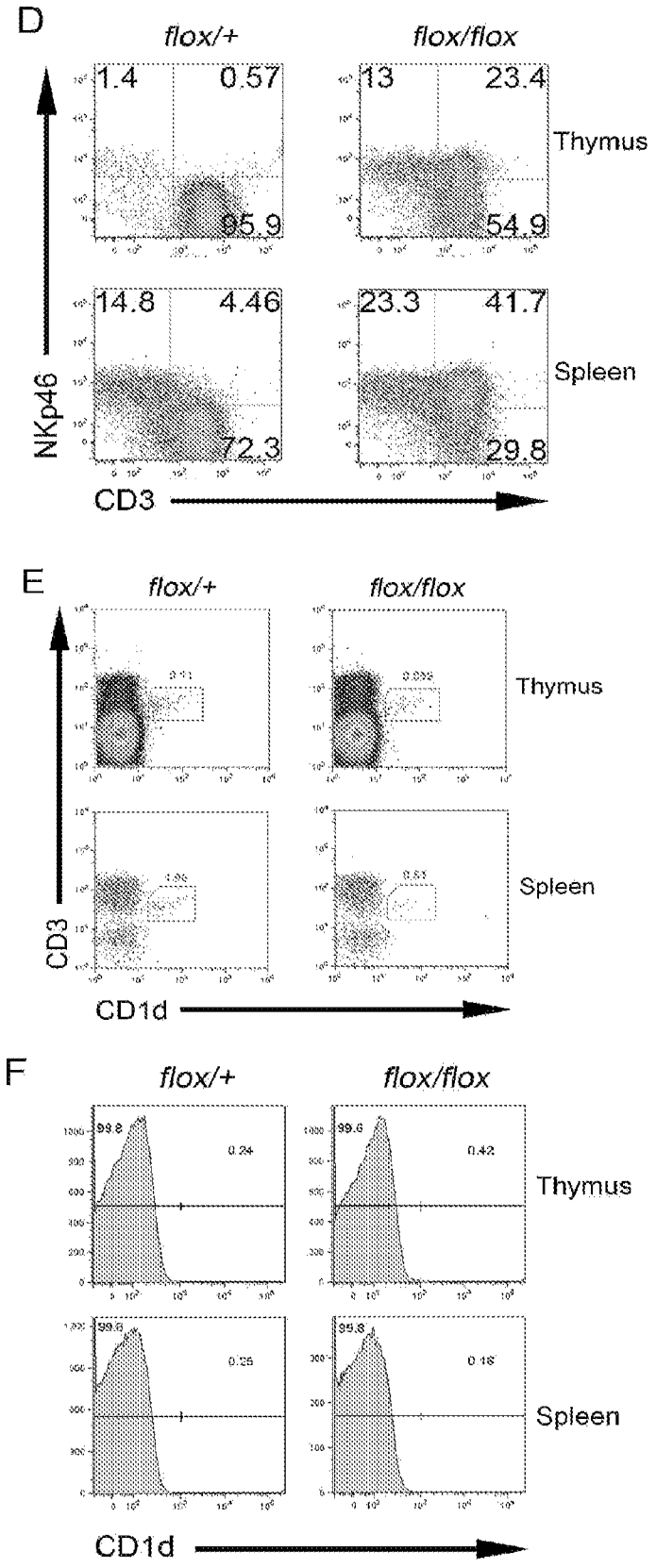
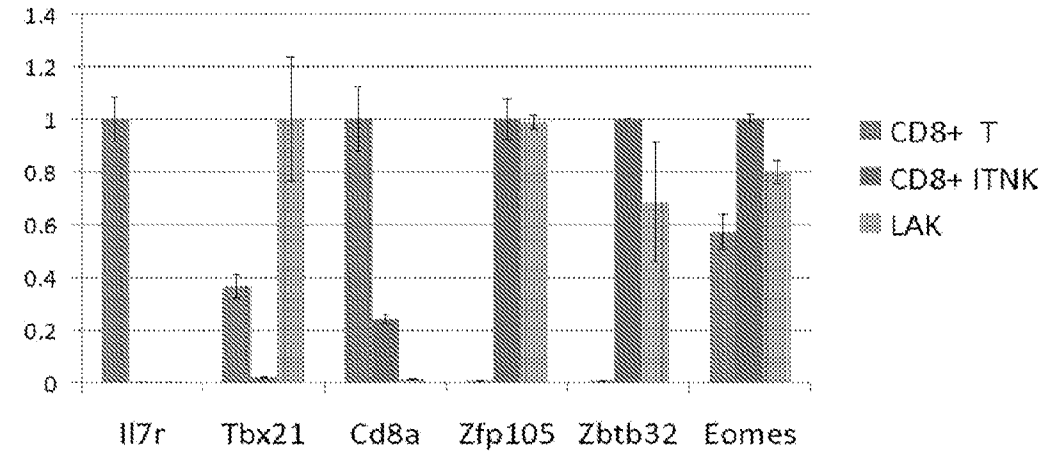
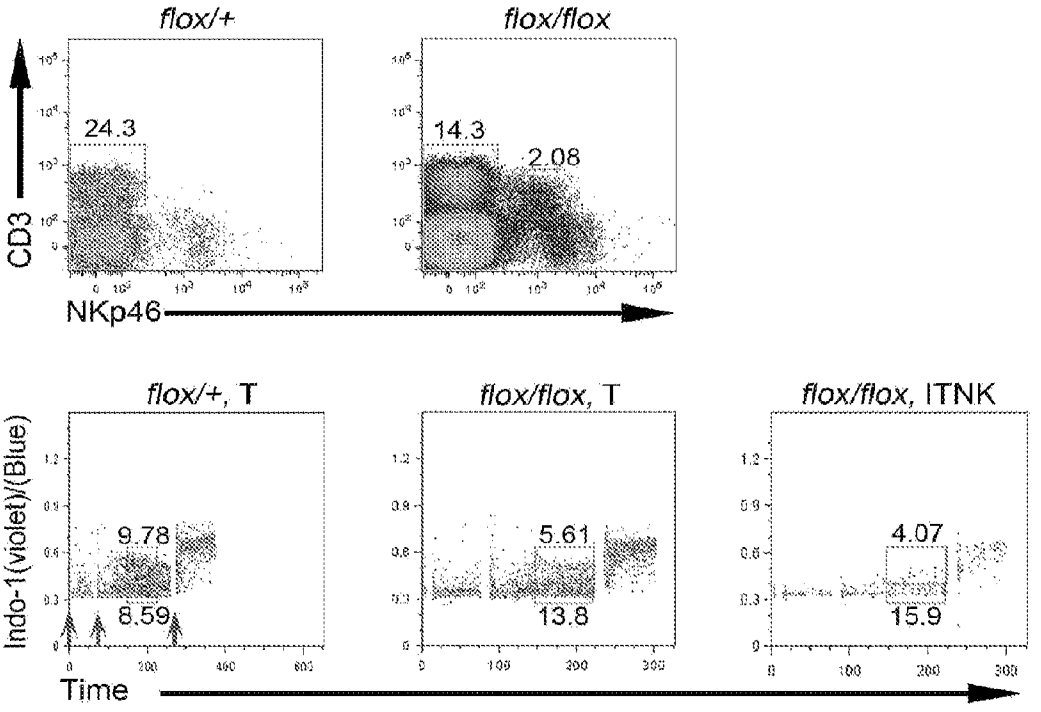

D
flox/+
flox/flox
1.4
0.57
95.9
13
23.4
54.9
Thymus
14.8
4.46
72.3
23.3
41.7
29.8
Spleen
NKp46
CD3
E
flox/+
flox/flox
Thymus
Spleen
CD3
CD1d
F
flox/+
flox/flox
99.8
0.24
99.6
0.42
Thymus
99.6
0.26
99.8
0.16
Spleen
CD1d

Figure 10

G



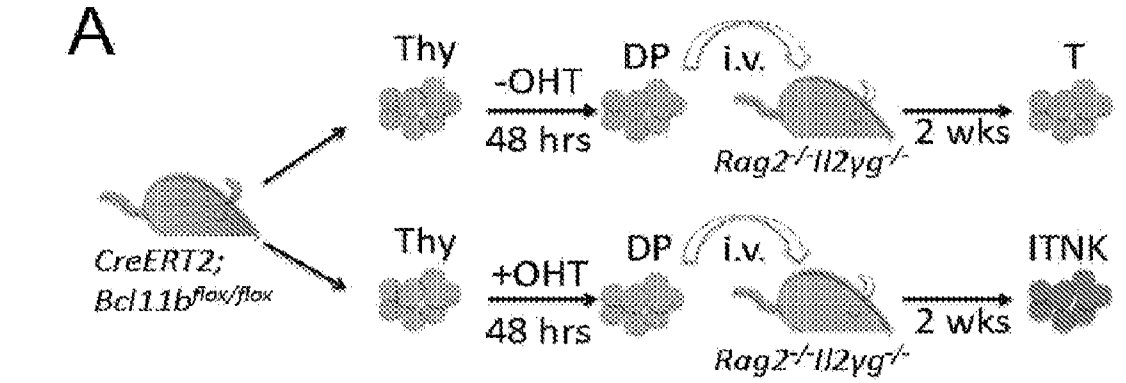
H

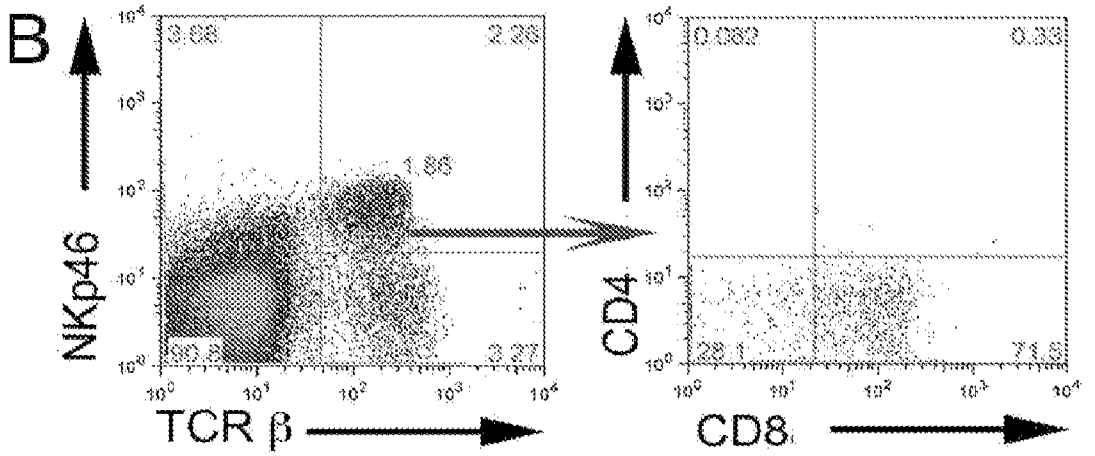


	flox/+, T	*flox/flox*, T	*flox/flox*, ITNK
Responding cells (%)	9.78	5.61	4.07
Non-responding cells (%)	8.59	13.8	15.9
Responders/Non-responders	1.14	0.416	0.256

Values are % of cells in gates indicated on plots in three panels above.

Figure 11


A
Thy
-OHT
48 hrs
DP
i.v.
T
2 wks
Rag2-/-Il2γg-/-
CreERT2;
Bcl11bflox/flox
Thy
+OHT
48 hrs
DP
i.v.
ITNK
2 wks
Rag2-/-Il2γg-/-


B
NKp46
TCR β
CD4
CD8
3.68
2.26
1.86
3.27
0.082
0.33
28.1
71.8

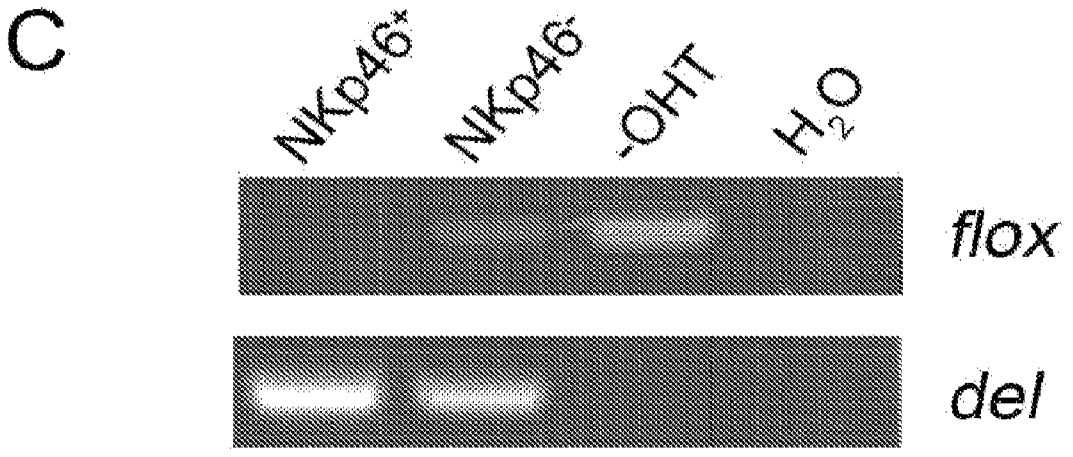
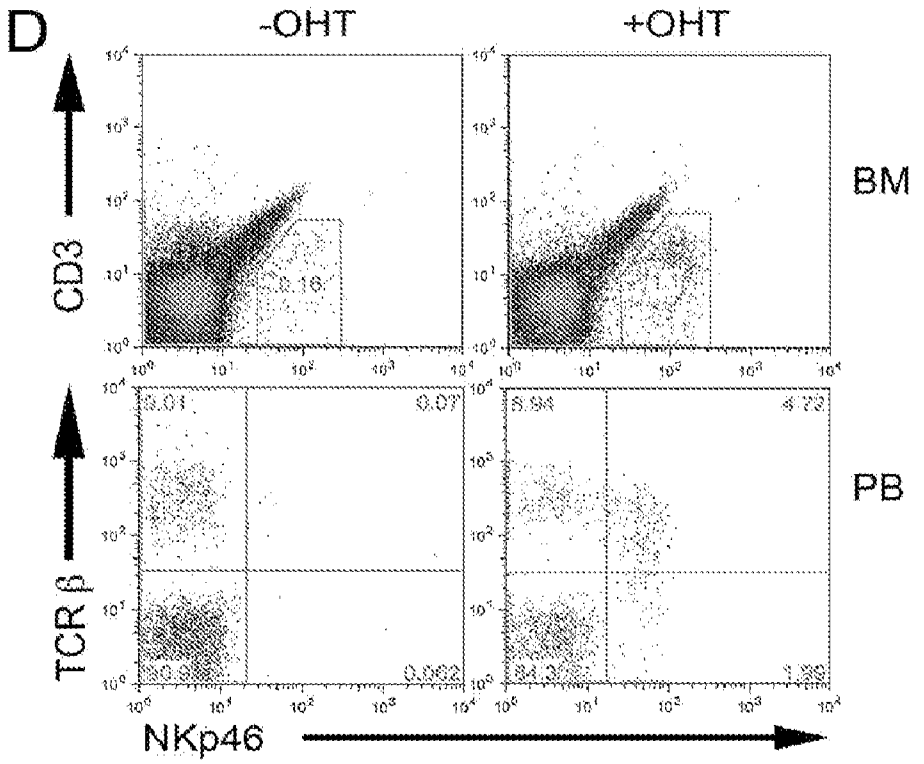

C
NKp46+
NKp46-
-OHT
H2O
flox
del

Figure 11


D
-OHT
+OHT
BM
PB
CD3
TCR β
NKp46
0.16
0.01
0.07
0.002

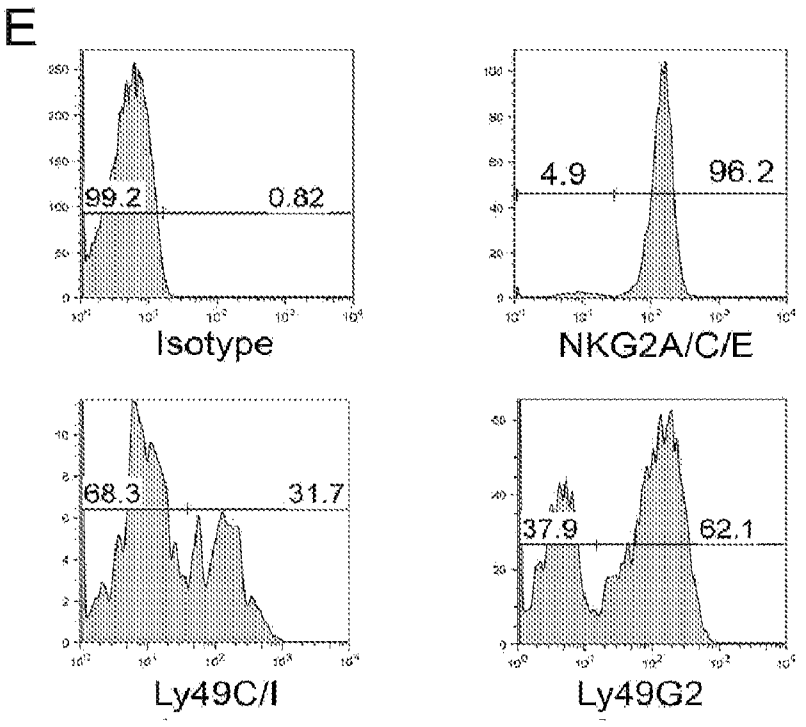

E
99.2
0.82
Isotype
4.9
96.2
NKG2A/C/E
68.3
31.7
Ly49C/I
37.9
62.1
Ly49G2

Figure 11

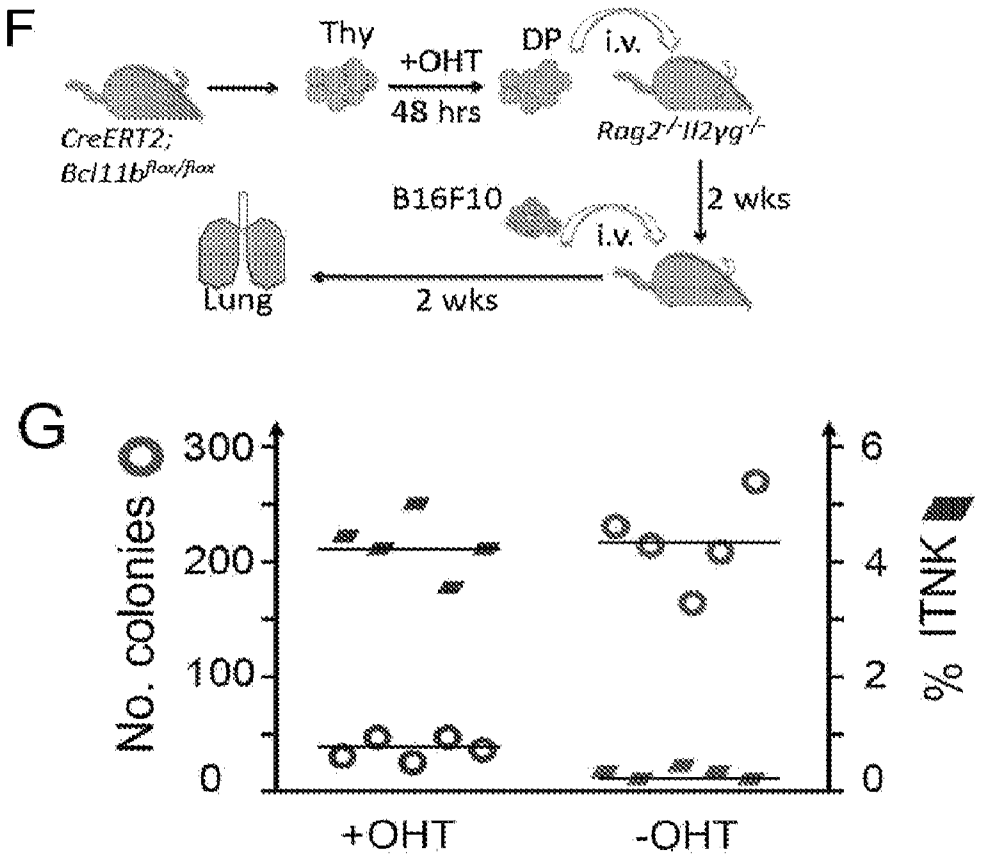


Figure 12

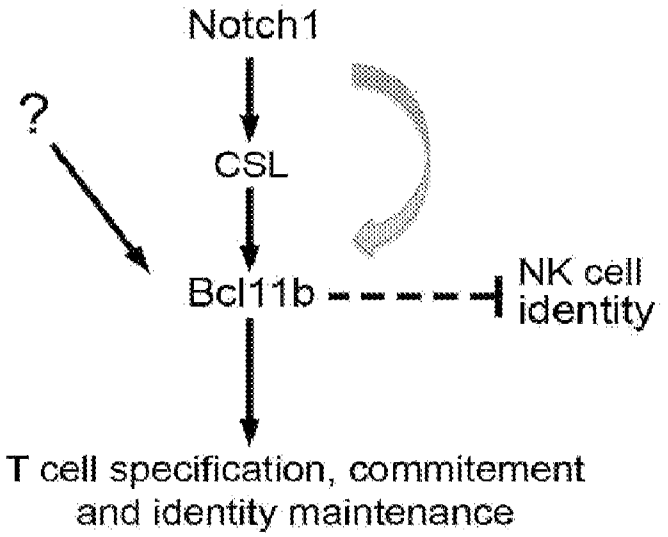


Figure 13

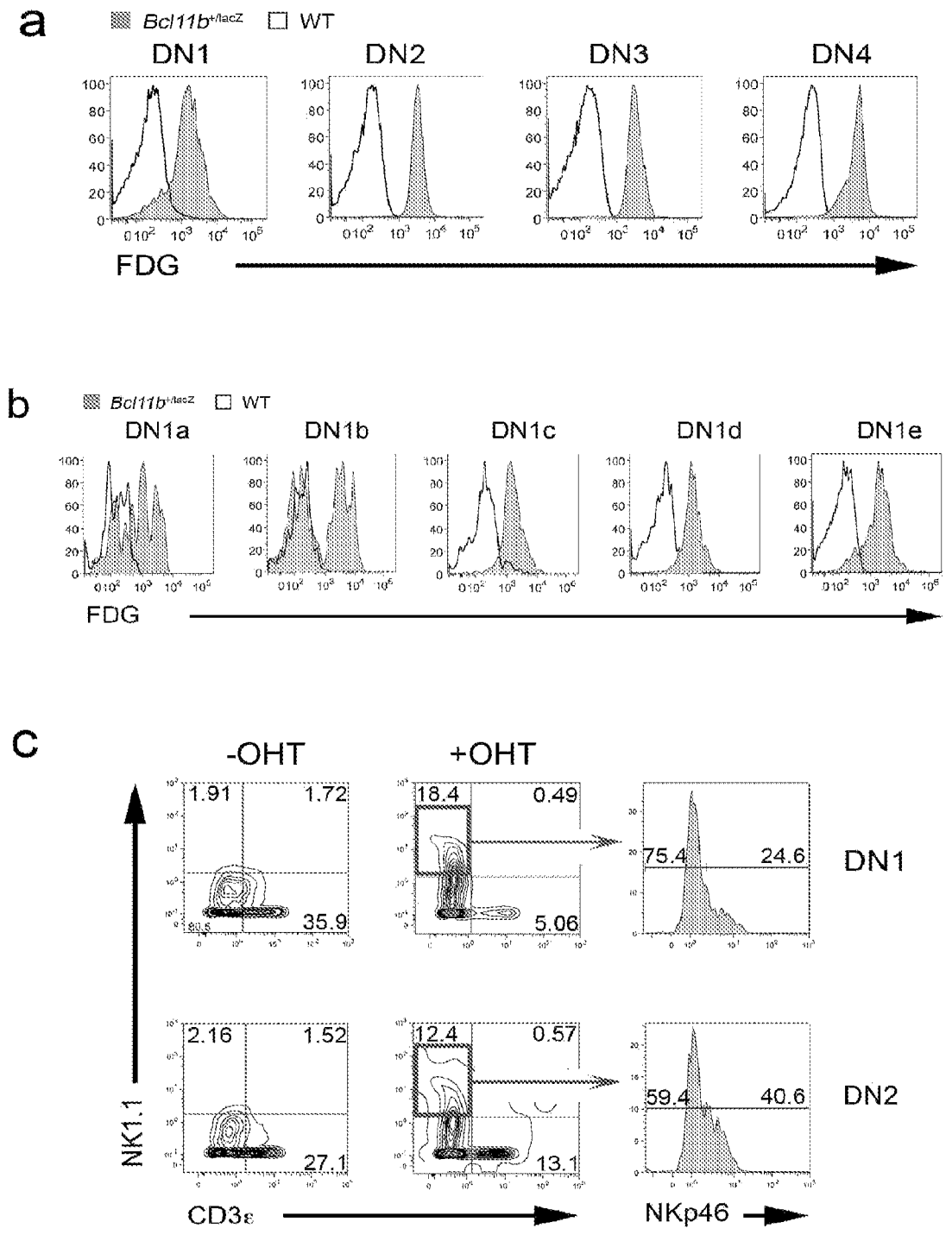
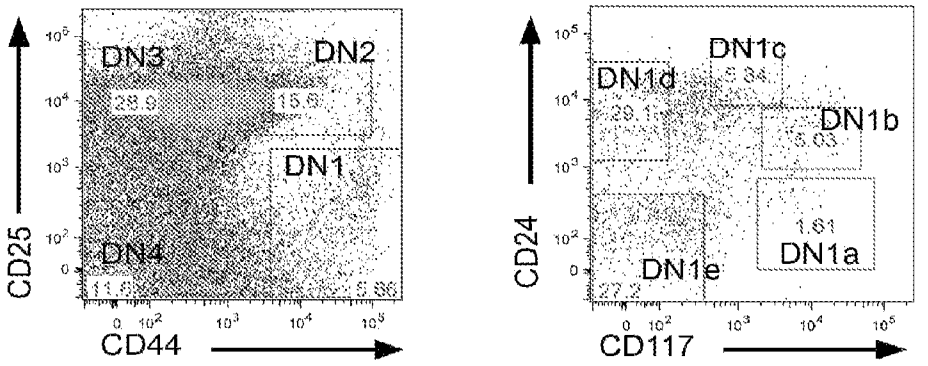

a
Bcl11b+/lacZ
WT
DN1
DN2
DN3
DN4
FDG
b
Bcl11b+/lacZ
WT
DN1a
DN1b
DN1c
DN1d
DN1e
FDG
c
-OHT
+OHT
1.91
1.72
35.9
18.4
0.49
5.06
75.4
24.6
DN1
2.16
1.52
27.1
12.4
0.57
13.1
59.4
40.6
DN2
NK1.1
CD3ε
NKp46

Figure 14

a


DN3
DN2
DN1
DN4
CD25
CD44
DN1c
DN1d
DN1b
DN1a
DN1e
CD24
CD117

b

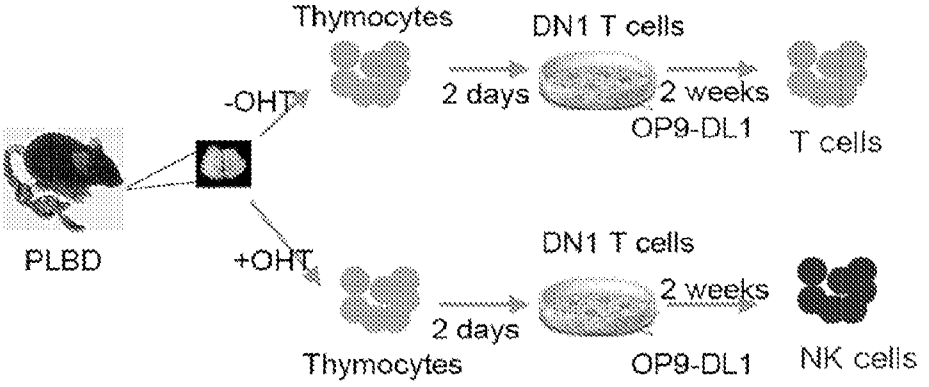
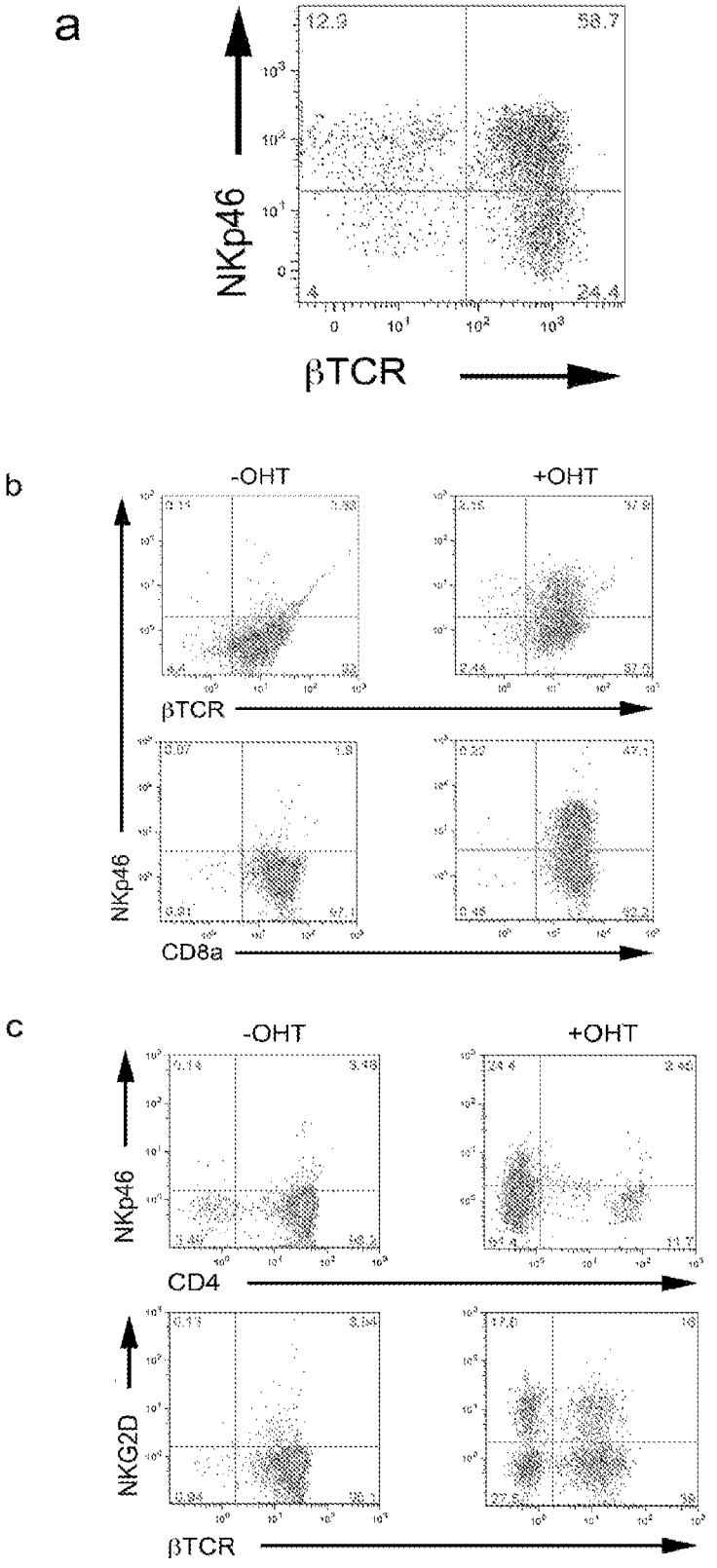

Thymocytes
DN1 T cells
2 days
2 weeks
OP9-DL1
T cells
-OHT
PLBD
+OHT
DN1 T cells
2 weeks
2 days
Thymocytes
OP9-DL1
NK cells

Figure 15


a
12.3
58.7
24.4
NKp46
βTCR
b
-OHT
+OHT
βTCR
NKp46
CD8a
c
-OHT
+OHT
NKp46
CD4
NKG2D
βTCR

CELLS, COMPOSITIONS AND METHODS

[0001] The present invention relates to induced T-to-Natural-Killer cells [herein "ITNK" cells], methods for their production and use of such cells, as well as methods for producing T cells.

[0002] Natural killer (NK) cells are a type of cytotoxic lymphocyte that constitute a major component of the innate immune system. NK cells play a major role in the rejection of tumors and cells infected by viruses and microbes. NK-cells are large granular lymphocytes (LGL) and constitute cells differentiated from stem cells or multipotent progenitors. The molecular mechanisms controlling the development of different cell types from stem cells is not fully understood.

STATEMENTS OF INVENTION

[0003] The invention provides a method of producing induced T-to-Natural-Killer [ITNK] cells from T cells and/or pro-T cells, the method comprising modulating the activity and/or effect of the Bcl11b gene and/or Bcl11b protein present in a T cell or pro-T cell, thereby converting said T cell and/or pro-T cell to an ITNK cell.

[0004] The invention provides a method of producing target T cells and/or target pro-T cells, the method comprising modulating the activity and/or effect of at least one Bcl11b gene and/or protein product present in a T cell and/or pro-T cell, and converting said T cell and/or pro-T cell to said target T cells and/or target pro-T cells.

[0005] The invention provides an ITNK cell obtainable, or obtained, from a T cell or pro-T cell. Suitably the T cell or pro-T cell includes a Bcl11b gene and/or gene product the activity and/or effect of which has been modulated so that the T cell or pro-T cell is capable of conversion to a ITNK cell.

[0006] The invention also relates to mature activated T cells in which Bcl11b expression is downregulated or absent (hereafter referred to as TBcl11b-cells), for use in medicine, such as prophylaxis or treatment of disease. The invention also relates to isolated or purified mature activated T cells in which Bcl11b expression is downregulated or absent.

[0007] The invention provides a target T cell or target pro-T cell obtainable, or obtained, from a T cell or pro-T cell respectively. Suitably the target cell comprises at least one Bcl11b gene and/or gene product the activity and/or effect of which has been modulated when compared to the wild type cell, so that the target T cell or target pro-T cell is capable of conversion to an ITNK cell. Wild type cells in the context of this disclosure does not refer to cancerous or transformed cells.

[0008] The invention provides a pharmaceutical composition comprising ITNK cells, or target T cells, or target pro-T cells together with a pharmaceutically acceptable excipient.

[0009] The invention provides ITNK cells or target T cells or target pro-T cells for use in therapy.

[0010] The invention provides a method of treating a human or non-human mammal subject suffering from, or susceptible to disease such as cancer or viral infection, the method comprising administering to the subject a therapeutically effective amount of ITNK cells or target T cells/pro-T cells, preferably ITNK cells or target T cells/pro-T cells which are derived from T cells or pro-T cells that have been obtained from that subject.

[0011] The invention provides a method of treating a human or non-human mammal subject suffering from, or susceptible to disease such as cancer or viral infection, the method comprising administering to the subject a therapeutically effective amount of a compound which modulates or inhibits the expression, activity and/or effect of Bcl11b gene or protein in T cells or pro-T cells and leads to the conversion of these T cells or pro-T cells to ITNK cells.

[0012] The invention provides an assay for identifying a target with which the Bcl11b gene product and/or protein product interacts or has an effect thereon, which assay comprises modulating the activity of a Bcl11b gene and/or gene product and monitoring the interaction or effect on a potential downstream target. Optionally a downstream target thus identified is modified to cause or assist in ITNK cell production.

[0013] The invention also relates to upstream modulators of Bcl11b activity, suitably those capable of causing or assisting in the conversion of T cells or pro-T cells to ITNK cells or target T cells/pro T cells. The invention also relates to methods for identification of upstream modulators of Bcl11b comprising identification of compounds that are able affect Bcl11b gene or protein expression or activity or effect, suitably as assessed by an effect of the upstream modulator on ITNK formation as disclosed herein.

[0014] In one aspect the invention relates to the use of factors which regulate the Bcl11b gene or protein expression or activity, or which are functionally downstream of the Bcl11b gene or protein, or which are functionally upstream of the Bcl11b gene, to effect the conversion of T cells to ITNK cells, and to the use of modulators of these factors to effect the conversion of T cells to ITNK cells.

[0015] The invention provides an assay for identification of a compound which assists in the reprogramming of T cells or pro-T cells to ITNK cells, the method comprising contacting T cells or pro-T cells with a test compound and monitoring or selecting for the conversion of T cells to ITNK cells or target T/pro T cells.

[0016] The invention provides an assay for identification of a mutation which results in or contributes to the reprogramming of T cells or pro-T cells to ITNK cells, the method comprising mutagenesis of T cells or pro-T cells and monitoring or selecting for the conversion of T cells to ITNK cells, followed by identification of the location of the mutation.

[0017] The invention provides an assay for identification of a compound which assists in the reprogramming of T cells to ITNK cells, the method comprising screening for compounds that bind to the Bcl11b DNA or RNA or the Bcl11b protein, and assessing whether said compounds are able to promote the conversion of T cells to ITNK cells.

[0018] The invention further provides use of compounds so discovered in the conversion of T cells or pro-T cells to ITNK cells.

[0019] The invention further provides a non-human animal carrying ITNK cells, and/or target T cells or target pro-T cells.

FIGURES AND TABLES

[0020] FIG. **1**. Bcl11b is essential for T cell development and for maintaining T cell identity.

(A) Flow cytometry profiles of cultured DN1 and DN2 thymocytes (+OHT) in the absence of IL-2.

(B) Flow cytometry profiles of cultured flox/flox DN3 thymocytes (±OHT) supplemented with IL-2.

(C) Killing of OP9-DLI stromal cells by OHT-treated flox/flox DN3 thymocytes.

(D) DNA from purified NKp46^{+} cells was prepared and subjected to PCR to detect DJ (top) and VDJ (bottom) recombination at the TCRβlocus.

(E-G) Microarray analysis of gene expression in NKp46$^+$ CD3$^+$ ITNK cells from DN3 thymocytes.

(E) Two-way hierarchical cluster map of the array data.

(F) and (G) qRT-PCR validation of gene expression of selected genes among ITNKs, LAKs and DN3 cells.

[0021] FIG. **2**. Efficient reprogramming of T cells to ITNKs.

(A) Representative flow cytometry profiles of ITNKs reprogrammed from single flox/flox DN3 cells.

(B) PCR genotyping of Bcl11b deletion in two representative T cell (T1, T2) and ITNK (I1, I2) wells.

(C) DJ recombination at the TCRβ locus of five ITNK wells (I1-I5) showing unique DJ recombination.

(D) Giemsa stain of parental DN3 thymocytes (T) and ITNK cells.

(E) and (e) Transmission electron micrographs of an ITNK cell.

(F) Cytotoxicity of ITNKs (labeled as "+OHT") and LAKs measured in standard ^{51}Cr release assays with B16F10, RMA and RMA-S tumor cell targets at the indicated effector-to-target (E:T) ratios. −OHT: flox/flox T cells.

[0022] FIG. **3**. ITNKs reprogrammed in vivo were potent tumour cell killers.

(A) Flow cytometric analysis of thymocytes and splenocytes from OHT treated flox/flox and flox/+ mice.

(B) Analysis of ITNKs from thymic γδ T cells in OHT treated flox/flox mice.

(C) ITNKs production in Rag2$^{-/-}$Il2rg$^{-/-}$ recipients injected with flox/flox DP thymocytes.

(D) Ex vivo expansion of ITNKs in IL-2 from splenocytes of the recipient mice.

(d) Ex vivo expansion of in vivo reprogrammed iTNK cells starting from splenotypes of four Rag2$^{-/-}$Il2γc$^{-/-}$ recipient mice.

(E) The ex vivo-expanded ITNKs (labeled as "+OHT") were used in ^{51}Cr release killing assays with B16F10, RMA and RMA-S tumor cell targets at the indicated effector-to-target (E:T) ratios. −OHT: flox/flox T cells.

(F) ITNKs prevented tumour metastasis. Rag2$^{-/-}$Il2rg$^{-/-}$ recipients transplanted with treated (+OHT) or untreated (−OHT) flox/flox DP thymocytes or PBS and subsequently injected intravenously with 50,000 B16F10 melanoma cells.

(G) In vivo iTNKs effectively eliminated B16F10 melanoma cells in mice.

[0023] FIG. **4**. Bcl11b is a direct downstream target gene of Notch signaling.

(A). Bcl11b protein in T cells following OHT treatment detected by Western blot.

(B) Schematic of the Bcl11b locus showing putative CSL binding sites (BS) and that of an irrelevant control binding site (CTL).

(C) Genomic DNA was prepared from immunoprecipitation of thymocytes, using CSL or control IgG antibodies, and was amplified using primers flanking the putative CSL or the control binding sites at the Bcl11b locus.

[0024] FIG. **5**. Generation of the Bcl11b-tdTomato reporter mouse.

(A) The tdTomato cassette was targeted to the 3' UTR of the Bcl11b locus.

(B) Insertion of the tdTomato cassette at the Bcl11b 3' UTR did not affect T cell development.

[0025] FIG. **6**. Detection of Bcl11b expression in hematopoietic lineages using the Bcl11b-tdTomato reporter mice.

(A) CD4 CD8 double negative (DN; DN1-DN4) thymocyte subsets.

(B) Double positive (DP) thymocytes (CD4$^+$CD8$^+$), splenic CD4$^+$ and CD8$^+$ T cells, thymic γδ T cells, and splenic NKT cells (CD3$^+$CD1d$^+$).

(C) Bone marrow B cells (CD19$^+$B220$^+$) and myeloid cells (CD11b$^+$Gr-1$^+$).

(D) Splenic (CD3), and thymic (CD3CD4CD8) NK cells.

(E) qRT-PCR of Bcl11b expression in sorted splenic naïve (CD44$^-$CD62L$^+$) and activated (CD44$^+$CD62L$^-$) T cells population.

(F) Quantification of Bcl11b expression in naïve and activated T cells in the Bcl11b$^{td/+}$ mice.

[0026] FIG. **7**. Strategies for identification of cell populations for flow sorting and analysis.

(A) Identification of double negative (DN) thymocyte (DN1-DN4) populations defined by Lin and expression of CD25 and CD44.

(B) Identification of γδ T cells.

(C) Identification of NKT cells in the spleen by first gating (or, prior to FACS sorting, magnetically depleting) out B cells.

(D) Identification of NK precursors and NK cell subsets cells.

(E) Thymic NK cells were defined as NK1.1$^+$CD127$^+$ thymocytes.

(F) Identification of naïve (CD44$^-$CD62L$^+$) and activated (CD44$^+$CD62L$^-$) T cells.

[0027] FIG. **8**. In vitro analysis of Bcl11b-deficient T cells.

(A) Schematic diagram of the Bcl11b conditional knockout allele. (B) Experimental design for the analysis of Bcl11b-deficient DN thymocytes. (C) NKp46$^+$CD3 cells from DN1 and DN20HT-treated flox/flox thymocytes did not express TCRβ.

(D) Homozygous Bcl11b deletion in ITNK (NKp46$^+$CD3) but not in T (NKp46$^-$CD3$^+$) cell populations from DN1 and DN2 cultures.

(E) No NKp46$^+$ cells but T cells were obtained from untreated flox/flox thymocytes.

(F) NKp46$^+$TCRβ$^-$ cells from OHT-treated DN1 and DN2 flox/flox thymocytes in the absence of IL-2 or IL-15 cultured on OP9 stromal cells.

(G) NKp46$^+$TCRβ$^-$ cells were detected in OHT-treated DN3 flox/flox, but not flox/+, thymocytes in T cell media.

(H) Reprogramming of Bcl11b-deficient DN3 thymocytes to NKp46$^+$ cells in myeloid cell culture condition.

(I) Reprogramming of Bcl11b-deficient DN3 thymocytes to NKp46$^+$CD19$^-$ cells in B cell culture condition.

(J) Venn diagram comparison of the upregulated (>2-fold) genes between LAK vs DN3 (green) and ITNK vs DN3 (purple).

(K) ITNKs from DP flox/flox thymocytes treated with OHT and cultured on OP9-DL1 in the presence of IL-2.

(L) ITNKs from splenic flox/flox CD8$^+$ T cells treated with OHT cultured on OP9-DL1 in the presence of IL-2.

[0028] FIG. **9**. Characterization of in vitro reprogrammed ITNK phenotype.

(A) and (a) Experimental design for reprogramming of single DN3 thymocytes to ITNK.

(B-C) Expression of intracellular and NK cell surface markers by the reprogrammed ITNK from DN3 thymocytes in vitro.

(D) Expression of NK cell markers by ITNKs reprogrammed from Bcl11b-deficient DP thymocytes in vitro.

(E) ITNKs did not express CD127 and thus were not thymic NK cells.
(F) Analysis of CD27 and CD11b in bulk-cultured ITNKs reprogrammed from DN3 thymocytes.

[0029] FIG. **10**. Analysis of in vivo reprogrammed ITNK cells in the flox/flox mouse.
(A) Experimental design for the analysis of in vivo reprogrammed ITNK cells.
(B) PCR of Bcl11b deletion in ITNK ($NKp46^+CD3^+$ and $NKp46^+CD3^-$) cell populations in flox/flox mice.
(C) Flow cytometric analysis of CD4 and CD8 expression in $NKp46^+$ ITNKs.
(D) Flow cytometric analysis of cells following ex vivo expansion of whole thymocytes or splenocytes from OHT treated mice.
(E) Flow cytometric analysis of CD1d-restriced NKT cells in thymus and spleen.
(F) Analysis of CD1d-restricted cells in the ex vivo-expanded ITNK culture.
(G) qRT-PCR analysis of several key T or NK cell-associated genes in $CD8^+$ T cells, $CD8^+$ ITNKs and LAKs.
(H) Splenocytes from flox/flox or flox/+ mice treated with Tamoxifen were stained with NKp46, NK1.1, CD8 and CD3 to confirm expression of CD3 on ITNKs.

[0030] FIG. **11**. In vivo reprogrammed ITNKs from DP thymocytes prevented tumour metastasis.
(A) Experimental design for the analysis of in vivo reprogramming of DP thymocytes to ITNKs.
(B) Most ITNKs in the spleen were $CD8^+$.
(C) ITNKs had complete Bcl11b deletion whereas donor derived NKp46 cells still retained at least one copy of the foxed allele. (D) ITNKs were also found in bone marrow and peripheral blood.
(E) Expression of additional NK cell surface markers on the in vivo reprogrammed ITNKs.
(F) ITNKs prevented tumour metastasis. $Rag2^{-/-}Il2rg^{-/-}$ recipients were transplanted with treated (+OHT) or untreated (−OHT) flox/flox DP thymocytes or PBSand subsequently injected intravenously with 5×10^4B16F10 melanoma cells.
(G) Plot shows inverse correlation between the percentage of ITNK cells (squares) obtained from recipient mice following in vivo reprogramming and tumor challenge and the number of lung colonies (circles) observed.

[0031] FIG. **12**. A working model showing that Bcl11b acts downstream of Notch signaling and promotes T cell development and maintains T cell identity.

[0032] FIG. **13**.

[0033] a. Expression of Bcl11b in thymocytes from Bcl11b-lacZ knock-in mice using the fluorescent substrate FDG.

[0034] b. Detection of Bcl11b expression in the five DN1 subpopulations.

[0035] c. Top, acute loss of Bcl11b caused DN1 thymocytes to express NK-specific genes. Bottom, deleting Bcl11b in DN2 thymocytes gave rise to the same phenotype of converting to NK-like cells.

[0036] FIG. **14**.

[0037] a. Left panel: different double negative (DN) thymocyte populations; Right panel: five subpopulations of DN1 thymocytes.

[0038] b. Flow chart of analyzing Bcl11b-deficient DN1 thymocytes.

[0039] FIG. **15**.

[0040] a. Double positive (DP) thymocytes expressed NKp46 after Bcl11b deletion.

[0041] b. Purified CD8 single positive cells (−OHT) proliferated on OP9-DL1 stromal cells. They did not express NKp46. Once Bcl11b was deleted, 38% of the cells now expressed NKp46 which killed the stromal cells.

[0042] c. Purified CD4 single positive cells (−OHT) growing in T cell media (left). Bcl11b deletion (+OHT) caused these CD4 T cells to express NKp46 and NKG2D.

Table 1. Comparison of gene expression profiles of ITNK, DN3 and LAK cells in microarray analysis.
Table 2. Comparison of cell surface receptor repertoire of LAK and ITNKs.
Table 3. Changes of gene expression profiles in thymocytes at 24 hours and 48 hours after deletion of Bcl11b in microarray analysis.
Table 4. PCR primers

GENERAL DESCRIPTION

[0043] T cells develop from early T cell progenitors which have NK and myeloid potential through a series of steps, known as DN1 (double negative stage 1), DN2, DN3 and DN4, DP (double positive), and then into single positive (SP) mature CD4 or CD8 positive T cells. There are many different types of T cells including helper, cytotoxic and regulatory T cells.

[0044] Activation of T cells is brought about by interaction with appropriate antigen MHC complex. For example, helper T cells become activated when they are presented with peptide antigens by MHC class II molecules that are expressed on the surface of Antigen Presenting Cells (APCs). The process of activation of T cells is known to the skilled person.

[0045] In the present invention we show that modulation of the Bcl11b gene/gene pathway allows T cells and pro-T cells to be reprogrammed into induced T-to-Natural-Killer (ITNK) cells. Data is presented for DN, DP and SP T cells. In addition, we show that such ITNK cells are effective in the amelioration of disease in an in vivo model and do not shown any adverse effects on the animal model. The Bcl11b protein in mice and humans is highly conserved, also, T cell development in both humans and mice is very similar. This information indicates that findings in mice may be extrapolated to the treatment or prevention of human diseases.

[0046] Reference to Bcl11b herein includes any Bcl11b homologues that may be identified in other species, suitably homologues that when deleted in whole or in part can result in the generation of ITNK cells in that species.

[0047] The invention provides a method of producing induced T-to-Natural-Killer [ITNK] cells from T cells and/or pro-T cells, the method comprising modulating the activity and/or effect of at least one Bcl11b gene and/or gene product present in a T cell or pro-T cell, thereby converting said T cell and/or pro-T cell to an ITNK cell.

[0048] The invention provides a method of producing target T cells and/or target pro-T cells, the method comprising modulating the activity and/or effect of at least one Bcl11b gene and/or protein product present in a T cell and/or pro-T cell, and converting said T cell and/or pro-T cell to said target T cells and/or target pro-T cells.

[0049] Reference to T cells includes, for example, DN, DP or SP T cells such as DN1, DN2, DN3, DN4, DP thymocytes, CD4 or CD8 single positive mature T cells or γδ-T cells.

Reference to pro-T cells includes common lymphoid precursor cells, stem cells and other non-T hematopoietic cells or non-hematopoietic cells which can be converted to T cells

[0050] Target T cells or target proT cells are cells which have the potential to convert into ITNK cells as a result of the modulation of the activity and/or effect of at least one Bcl11b gene and/or gene product in the T cell or pro T cell, but which have not yet converted to give the ITNK like phenotype.

[0051] Modulation of the activity or the effect of the Bcl11b gene or protein is suitably achieved by inhibiting the activity or effect of Bcl11b, either directly or indirectly.

[0052] Suitably the inhibition comprises deletion of at least part of said Bcl11b gene, suitably at least a single exon of the Bcl11b gene, suitably at least exon 4 of the Bcl11b gene. In one aspect all of the gene is deleted. Suitably, inhibition of the activity or effect of Bcl11b may be achieved by disrupting the function of Bcl11b through insertion a genetic cassette to the Bcl11b locus. Suitably, inhibition of the activity or effect of Bcl11b may be achieved by modulating epigenetic changes at the Bcl11b locus or those gene loci that regulate Bcl11b or are regulated by Bcl11b. Suitably, inhibition of the activity or effect of Bcl11b may be achieved by using antibodies (conventional or peptide Abs) to neutralize gene products of Bcl11b or its upstream or down-stream genes.

[0053] In another aspect the invention relates to genomes comprising a Bcl11b conditional knockout (cko) allele, preferably T cells or pro T cells having such a conditional mutation. The generation of conditional alleles allows the growth of cells under conditions in which Bcl11b is expressed, followed by growth under different conditions that cause the Bcl11b gene to be deleted and the ITNK phenotype to be expressed. Thus the invention also relates to a process for the induction of ITNK cells comprising activation of a conditional mutation, suitable to modulation of the activity and/or effect of at least one Bcl11b gene and/or gene product in the T cell or pro T cell.

[0054] In one aspect the modulation is directly at the level of Bcl11b gene expression, where the expression of Bcl11b is preferably inhibited to stimulate ITNK cell production. In one aspect the sequences of the Bcl11b gene, or control sequences such as promoter or enhancer regions, may be mutated, such that transcription or translation are adversely affected.

[0055] In one aspect control of the expression of Bcl11b is achieved by control of mRNA expression or protein translation. In one aspect the expression of Bcl11b is modulated by antisense RNA or the use of small interfering RNA (sRNA) or miRNA.

[0056] In one aspect modulation of Bcl11b is at the protein level. The activity of the Bcl11b protein may be modulated, preferably inhibited, by Bcl11b binding proteins, for example.

[0057] In one aspect modulating or inhibiting of the activity and/or effect of said Bcl11b gene or protein produces a downstream modulation in a biological pathway (s) in which said Bcl11b protein is involved. In one aspect said downstream modulation regulates the presence and/or activity and/or effect of a downstream target in said biological pathway. Assessment of downstream elements regulated by Bcl11b allows alternative targets to be identified which may control ITNK production from T cells and pro-T cells. The present invention also relates to identification of downstream targets—see below.

[0058] The invention provides an ITNK cell obtainable, or obtained, from a T cell or pro-T cell, including from stem cells or progenitors, wherein the T cell or pro-T cell includes a Bcl11b gene and/or gene product the activity and/or effect of which has been modulated so that the T cell or pro-T cell is capable of conversion to a ITNK cell.

[0059] The invention also provides a target T cell or target pro-T cell including at least one Bcl11b gene and/or gene product the activity and/or effect of which has been modulated when compared to the wild type cell, so that the T cell or pro-T cell is capable of conversion to an ITNK cell. The target T cell or target pro-T cell may be an ES cell, or adult stem cell, or induced pluripotent stem cell (IPS cell).

[0060] In one aspect of the invention the ITNK cells or target T/pro T cells are obtained from T cells or pro-T cells in which all or part of the Bcl11B gene has been deleted. In one aspect there is a deletion in both alleles of the Bcl11b gene, or part thereof.

[0061] The invention also relates to a mammalian genome from which all or part of the Bcl11b gene has been deleted.

[0062] The invention also relates to mature activated T cells in which Bcl11b expression is downregulated or absent (also referred to as TBcl11b-cells). Mature T cells in this context refer to normal mature T cells and not to cancerous or transformed T cells. As shown in the example section below, it has been observed by the present inventors that at a single cell level about 10-20% of activated splenic T cells have very low level of Bcl11b expression (also FIG. **6** (F)). Hence, use of these cells in medicine, particularly in the treatment of cancers and viral infections forms an aspect of this invention.

[0063] The invention also relates to cells, such as T cells and pro T cells and stem cells and animals such as non-human animals, such as a mouse, the genome of which comprises a Bcl11b conditional knockout (cko) allele.

[0064] In one aspect all or part of Bcl11b gene is floxed or otherwise associated with recombinase target sequences, to allow the Bcl11b gene or part thereof to be deleted. In one aspect the cell comprising the floxed gene expresses Tamoxifen (OHT)-inducible Cre recombinase. Expression of the Cre recombinase by OHT induction suitably causes all or part of Bcl11b to be deleted.

[0065] The invention also relates to a cell or non-human mammal in which the Bcl11b gene or protein activity has been modulated, other than by deletion, to produce an ITNK cell or target ITNK cell.

[0066] ITNK cells suitably are obtained or obtainable from another cell type (such as T cells or pro-T cells, suitably DN1, DN2, DN3, DN4, DP thymocytes, CD4 or CD8 single positive mature T cells, common lymphoid precursor cells or stem cells) and suitably exhibit one or more or all of the following properties:

[0067] (a) a morphology comparable to natural killer cells, in comparison to T cells, for example as shown in FIG. **2**D, FIG. **2**E and FIG. **2***e*.

[0068] As shown below, reprogrammed thymocytes not only expressed NK cell surface receptors but morphologically do not look like T cells, rather, they were much similar to regular NK cells which are large size, large cytoplasm, have granules and high protein synthesis activity in the abundant endoplasmic reticulum (ER) (FIGS. **2**D, **2**E and **2***e*).

[0069] (b) TCR 6 specific genomic DNA re-arrangement, for example as shown in FIG. **2**C;

[0070] As shown below, certain ITNK cells have a rearranged TCR 6 locus, indicative of their origin as T cells.

[0071] (c) a gene expression profile more similar to that of NK cells, such as LAK cells, than the parental cells from

which they were developed, for example as shown in FIGS. **1**E and **1**G. Genes that showed an expression difference between the parental DN3 thymocytes and their Bcl11b-deficient derivatives are listed in Table 1. When considering this table of genes, ITNK cells suitably have at least 50%, suitably at least 60%, suitably at least 70% of genes differentially expressed (2 fold difference or more) in the same direction (increase or decrease) as LAK cells.

[0072] (d) cellular expression of one or more NK specific genes not found, or not expressed at high levels on non-effector or naïve T cells such as:

[0073] ZFP105, IL2Rβ, Id2, JAK1, NKG2D, NKG2A/C/E, B220, Rog (Zbtb32), Tnfrsf9, Cdkn1c, Trail, Perforin, Interferon-γ, NK1.1, NKp46, E4 bp4, NKG7, KLRD1, LTA, PLCG2, Ly49C/I and Ly49G2

[0074] (e) decreased or no expression of one or more T lineage genes, in comparison to the parent cells from which the ITNK cell was derived, such as decreased or no expression of Notch1, Est1, Hes1, Gata3, Deltaxi, TCRβ, CD3, Tcf1, IL7Ra, T-bet, CD8. In one aspect, ITNK cells are derived from CD8+ cells and do not express IL7R and/or T-bet and express low levels of CD8a.

[0075] (f) cell killing ability, for example the ability to prevent or ameliorate tumour formation or growth, the ability to kill stromal cells, tumour cells, or infected cells, suitably in comparison to the precursor cell used (parent T cells or proT cells). Cell killing may be assessed in vitro or in vivo by methods described in the Examples section herein. Additionally, the ITNKs can recognize MHC—I molecules. Moreover, the ITNK cells produced in vivo are not MHC—I restricted and are capable of killing MHC—I positive or negative cells. The ITNK cells whether produced in vitro or in vivo kill MHC—I low or negative cells.

[0076] (g) a mutation in the Bcl11b gene, or control sequences, affecting transcription, or translation or protein sequence, or otherwise affecting Bcl11b activity or effect, suitably promoting ITNK production.

[0077] Suitably the cells are capable of killing OP9-DL1 stromal cells, suitably within 2-20 days, such as 5-15 days such as 10 days after treatment to initiate the conversion from T cells or pro-T cells to ITNK cells, such as by OHT treatment. Suitably ITNKs retain a killing ability even when cultured in vitro for one month.

[0078] For the avoidance of doubt, ITNK cells produced by modulating Bcl11b activity and/or effect in a T cell and/or pro-T cell, remain ITNK cells according to the invention, if they retain cell killing ability even if Bcl11b returns to normal levels in such cells subsequently.

[0079] Suitably, ITNK cells of the invention exhibit the properties in (a), (c), (d), (e) and (f) above. Suitably, ITNK cells of the invention exhibit the properties in (a) or (c) or (d) or (e) and (f) above. ITNK cells may also possess one, or more, or all, of the following properties.

[0080] Suitably the proliferation and/or differentiation of the ITNK cells is promoted by a Supplement of IL-2 or IL-15 in the culture media.

[0081] Suitably ITNKs are able to grow out from T cell cultures within 2-20 days, such as 5-15 days, such as 10 days after Bcl11b is deleted or otherwise affected, or the Bcl11b pathway modulated suitably as assessed by the abundance of $NKp46^+$ cells (FIG. **8**K, **8**L, **15***a* and **15***b*).

[0082] Suitably T cell/pro T cell to ITNK cell conversion from T cells/pro-T cells is greater than 50% efficient, such as greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 95% efficient, suitably 100% efficient, by which it is meant that more than e.g. 50% of all cells in which the Bcl11b gene has been deleted, or in which the Bcl11b pathway has been otherwise modulated, go on to produce ITNK cells.

[0083] Suitably ITNK cells produced in vivo are detectable in the recipient host, such as a recipient mouse, for at least 1 month, preferably 2 months, preferably 3 months. Suitably recipient animals do not show any noticeable abnormality, indicating that the ITNK cells do not attack normal host cells in the recipient mice.

[0084] Suitably ITNK cells according to the invention possess functions of NK cells relating to regulation of the immune response, such as cytokine release.

[0085] Suitably ITNKs are able to continue proliferating for at least 3 weeks in cell culture.

[0086] In one aspect ITNK cells do not express NKp46.

[0087] Suitably ITNK cells or T cells can be independent of Notch signalling.

[0088] In one aspect the ITNK cells are not completely identical to NK cells. In one aspect ITNK cells do not express Ly49D. In one aspect ITNK cells do not express one or more T cell surface markers such as CD8, CD3e, and βTCR.

[0089] In another aspect ITNK cells express at least 20% of NK cell specific markers listed in table 2 as specific to LAK, preferably 40%, 60% or 80% of these known NK cell markers.

[0090] In one aspect, the ITNK cells produced in vivo are not MHC—I restricted and are capable of killing MHC—I positive or negative cells. The ITNK cells whether produced in vitro or in vivo kill MHC—I low or negative cells. This is explained in further detail in the example section below and shown in FIG. **3**E where it is shown that unlike LAK, the in vivo produced ITNK cells killed RMA cells with almost the same efficiency as killing RMA-S. Such in vivo produced ITNKs have the advantage that their use has no risk of autoimmune diseases.

[0091] In one aspect the ITNK cells have at least 2, 3, 4 or more of the properties listed above, and preferably all such properties.

[0092] In one aspect ITNK cells demonstrate a rearranged TCR β locus, do not express all of the genes listed in the table 2 as specific to LAK, and exhibit cell killing as described herein.

[0093] In one aspect the invention provides an ITNK cell obtainable or obtained by the present invention having by a cell killing ability as assessed by methods such as those of examples 1.1.9 and 1.1.11 herein, but which do not express Ly49D.

[0094] In one aspect the NK cells comprise a suicide gene or other mechanism to allow ITNK cells to be eliminated. By way of example the genome of the ITNK cell, or T cell or pro-T cell may be engineered to contain a negative selection cassette.

[0095] The invention provides a pharmaceutical composition comprising ITNK cells together with a pharmaceutically acceptable excipient. Suitable excipients are well known in the art and include pharmaceutically acceptable buffers, preservatives, diluents and carriers and the like.

[0096] Also provided are mixtures of the ITNK cells of the invention with therapeutic agents such as anti-cancer agents or anti-infective agents e.g antiviral agents. The ITNK cells may be used in a combined preparation for simultaneous, separate or sequential use in disease therapy such as antican-

cer or antiviral therapy, although the use of ITNKs is not limited to cancer and antiviral therapy, and ITNKs might be useful for eliminating many types of abnormal cells. For example, ITNKs may also be used for treatment or prophylaxis of bacterial, yeast and parasite infections.

[0097] Suitable anticancer agents include alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other drugs affect cell division or DNA synthesis and function in some way. Other drugs include targeted therapies such as monoclonal antibodies and tyrosine kinase inhibitors and nanoparticles. Furthermore, also suitable are drugs that modulate tumor cell behaviour without directly attacking those cells, such as hormone treatments, known as an adjuvant therapy. As an alternative, agents for immunotherapy may also be included, such as use of interferons and other cytokines to induce an immune, and vaccines to generate specific immune responses.

[0098] Suitable anti-infectives include drugs that act to block viral entry into cells, drugs that prevent virus replication, such as reverse transcriptase inhibitors, integrase inhibitors, Protease inhibitors, and drugs that prevent virus release into the body.

[0099] Delivery of cells and compositions of the invention may be by any suitable route of administration including enteral or parenteral, such as by injection or infusion, for example in a once a day, once a week, once a month, or other suitable schedule. Multiple or single rounds of treatment may be employed.

[0100] The invention relates to a method for the preparation of a medicament for a human or non-human mammal comprising taking a sample of T cells, and converting the T cells to ITNK cells as described herein, optionally then using said cells in a medicament for treatment. Optionally the method comprises dilution or otherwise selection of a single T cell, and optionally manipulation of the T cell genome prior to use as a medicament.

[0101] The invention provides ITNK cells and target T/pro-T cells for use in medicine, and use of ITNK cells and target T/pro-T cells in the preparation of a medicament for the treatment or prophylaxis of disease, such as cancer or viral infection. ITNKs may also be used for treatment or prophylaxis of bacterial, yeast and parasite infections.

[0102] The invention also provides mature activated T cells in which Bcl11b expression is down-regulated or absent (also referred to as TBcl11b-cells) for use in medicine, and use of such cells in the preparation of a medicament for the treatment or prophylaxis of disease, such as cancer or viral infection.

[0103] NK cells play a major role in the rejection of tumors and cells infected by viruses and the ITNK cells of the present invention demonstrate anti cancer properties in vitro and in vivo. In one aspect ITNK cells produced from T cells or pro T cells are used to treat diseases such as cancer and infectious diseases such as viral infections.

[0104] The ability to convert T cells or pro-T cells into ITNK cells and use of TBcl11b-cells allows therapies to be developed using a patient's own cells, which can be used in the same patient without rejection.

[0105] The invention thus relates to use of a therapeutically effective amount of ITNK cells derived from the T cells or pro-T cells of a patient in the treatment or prevention of infection or disease in that individual. In a further aspect the cells may be used in another individual.

[0106] The invention provides a method of treating a patient, the method comprising administering to said patient a therapeutically effective amount of ITNK cells or TBcl11b-cells preferably wherein the ITNK cells are derived from T cells or pro-T cells that have been obtained from the patient.

[0107] Target T cells or pro-T cells may also be employed as above, in place of ITNK cells.

[0108] In one aspect, T cells/pro-T cells or target T cells or target pro-T cells of the invention do not refer to cancerous or transformed T cells.

[0109] In one aspect the ITNK cells according to the invention are obtained by modulating Bcl11b activity and/or effect in transformed or cancerous T cells, such as T cells from lymphoma patients, which may have different levels of Bcl11b as compared to wild type cells. In this aspect, the transformed or cancerous T cells are the T cells/pro-T cells or target T cells or target pro-T cells capable of conversion to ITNK cells.

[0110] In one aspect ITNK cells do not show any adverse effects on the patient.

[0111] In one aspect, the invention provides a method of isolating naturally occurring mature activated T cells in which Bcl11b expression is downregulated or absent (TBcl11b-cells) from a patient, expanding the cells in vitro and administering to the patient a therapeutically effective amount of the TBcl11b-cells for treatment of conditions such as cancer and viral infections.

[0112] In one aspect, the invention provides a method of isolating T cells/pro-T cells from a patient (human or non-human); modulating the activity and/or effect of the Bcl11b gene and/or gene product so that the T cell or pro-T cell is capable of conversion to ITNK cells; administering to the patient a therapeutically effective amount of ITNK cells or target T cells or target pro T cells for treatment of conditions such as cancer and viral infections.

[0113] In one aspect the ITNK cells are derived from a single T cell which is converted into ITNK cells using the methods described herein. This process suitably allows for a T cell specific for an antigen of interest, such as a disease specific antigen, such as a viral or microbial antigen or such as a tumour-specific antigen, to be converted into an NK-like cells.

[0114] From a single T cell up to 0.5 million ITNKs can be obtained. This is a much higher number as compared to human NK cells where approximately 1600 cells can be produced by proliferation of a single NK cell.

[0115] The invention relates to modulation of Bcl11b directly, and also use of components of the Bcl11b pathway and modulators thereof in the production of ITNK cells.

[0116] An appreciation that T cells and pro-T cells can be converted to ITNK cells allows this conversion to be used as an assay for compounds that might be used to control the conversion process. Thus the invention relates to an assay for identification of a compound which assists in the reprogramming of T cells to ITNK cells, the method comprising contacting T cells or pro-T cells with a test compound and then monitoring or selecting for the conversion of T cells to ITNK cells. Such compounds could include small chemical molecules, proteins (including but not limited to growth factors, cytokines, antibodies) or nucleic acid based therapies, and libraries of any of these compounds. The invention also relates to use of compounds so identified in the conversion of T cells or pro-T cells to ITNK cells and additionally to those compounds per se.

[0117] In addition the invention relates to an assay for identification of a genetic mutation which controls the reprogramming of T cells to ITNK cells, the method comprising random or targeted mutation of T cells or pro-T cells and screening for ITNK cells or selection of ITNK cells under conditions where T cells or pro-T cells are not viable.

[0118] An appreciation that Bcl11b plays a role in the conversion of T cells and proT cells to ITNK cells allows the Bcl11b gene and protein to be used directly as probes to identify other components in the Bcl11b signaling pathway, which may then be tested for an effect on conversion of T cells to ITNK cells. Thus the invention relates to an assay for identification of a compound which assists in the reprogramming of T cells to ITNK cells, the method comprising screening for compounds that bind to the Bcl11b gene or the Bcl11B protein, and further optionally assessing whether said compounds are able to promote the conversion of T cells to ITNK cells. The invention further relates to use of compounds so identified in the conversion of T cells or pro-T cells to ITNK cells and those compounds per se.

[0119] In a yet further aspect the invention relates to the use of factors which regulate the Bcl11b gene or protein expression or activity, or which are functionally downstream of the Bcl11b gene or protein, or which are functionally upstream of the Bcl11b gene, to effect the conversion of T cells to ITNK cells, and to the use of modulators of these factors to effect the conversion of T cells to ITNK cells. Suitably, the modulators are antibodies targeting Bcl11b or factors which regulate the Bcl11b gene or protein expression or activity or downstream gene products or upstream gene products. Suitably, the modulators are administered to human or non-human diseased subjects.

[0120] For example, Notch is upstream of Bcl11b. In one aspect modulators of Notch signalling are used to effect a conversion of T cells and proT cells to ITNK cells.

[0121] CSI acts upstream of Bcl11b. In one aspect modulators of CSL are used to effect a conversion of T cells and proT cells to ITNK cells.

[0122] In another aspect the invention relates to an assay for identifying a downstream target for Bcl11b, the assay comprising monitoring the effect of modulating the Bcl11b gene and/or protein product on a putative downstream target. Such an assay may further comprise monitoring conversion of T cells or pro-T cells to ITNK cells when the downstream target per se has been modified. Such an assay may further comprise identifying a modulator which either interacts with said downstream target so as to modulate the activity and/or effect thereof, to result in the conversion of a T cell or pro-T cell to one or more ITNK cells.

[0123] The invention further provides for a non-human animal carrying ITNK cells, and/or target T cells or target pro-T cells.

[0124] In one aspect ITNK are independent of Notch signalling.

[0125] In a further aspect the invention relates to a method of stimulating T cell production, the method comprising modulating the activity and/or effect of at least one Bcl11b gene and/or protein present in a pro-T cell, such as a human or embryonic stem cell, or IPS cell. Suitably the method comprises stimulating the Bcl11b expression or activity.

[0126] An understanding of the importance of Bcl11b in the T cell maturation pathway suggests that manipulation of the Bcl11b gene or protein expression or activity can help to stimulate T cell production. The present invention thus relates to use of activators of the Bcl11b pathway, either upstream or downstream, in the stimulation of T cells production, either in vivo or in vitro, and use of T cells so produced in medicine.

EXAMPLES

[0127] T cells develop in the thymus and are critical for adaptive immunity. Natural killer (NK) lymphocytes constitute an essential component of the innate immune system in tumor surveillance and defense against microbes and viruses.

General Introduction to T and NK cell development

[0128] T cell development involves progenitor homing, lineage specification and commitment, and requires a complex interplay among key transcription factors (1, 2). The earliest populations of thymocytes, which lack T cell receptor (TCR) co-receptors CD4 and CD8 (double negative or DN cells) (28), can be further subdivided by cell surface markers as DN1-4 (29). The DN1 ($CD44^+CD25^-$) thymocyte population contains multipotent progenitors (30, 31) whereas DN2 thymocytes ($CD44^+CD25^+$) have NK and myeloid potential (30, 31). These non-T cell developmental potentials are lost in the DN3 ($CD44CD25^+$) thymocytes. DN4 thymocytes ($CD44^-CD25^-$) have undergone have undergone β-selection after successful Tcrβ gene rearrangement (32) and already initiated the process of differentiating to the $CD4^+CD8^+$ double positive (DP) stage (33, 34).

[0129] In the periphery, the cytokine IL-7 and the constant interaction of T cells with self peptide-MHC play a critical role in T cell maintenance (3). RT-PCR analysis indicates that many genes important for T cell commitment start to increase their expression in the transition from DN1 to DN2, with Bcl11b being the most upregulated transcription factor (4). In bony fish, Bcl11b is shown to be required for T cell precursor homing to the thymus (5). In the mouse, Bcl11b has critical roles in fetal thymocyte development and survival, and in positive selection and survival of double-positive thymocytes (6, 7).

[0130] NK cell committed precursors ($CD122^+$) differentiate from multipotent haematopoietic progenitors primarily in the bone marrow but differentiation can also occur in the thymus and secondary lymphoid tissues (35). These precursors give rise to $NKp46^+$ immature NK cells, which subsequently express additional receptors as they differentiate, including MHC receptors, NKG2A/C/E and Ly49s (36, 12). Besides their participation in innate immune responses, NK cells have recently been shown to possess some adaptive immune features (37).

[0131] Although NK developmental pathways are not entirely clear, two subsets of NK cells, bone marrow-derived ($CD127^-$) and thymic ($CD127^+$) NK cells have been identified in the mouse that differ in development sites and origins (Huntington et al., 2007). Previous studies have identified molecules important for NK cell development and homeostasis. For example, Id2, which antagonizes the bHLH E proteins E2A and HEB, is essential for the NK lineage since the Id2-knockout mice lack NK cells (Ikawa et al., 2001; Yokota et al., 1999). Conversely, forced expression of Id2 or Id3 is able to re-direct pro-T cells to NK cell differentiation (Blom et al., 1999; Fujimoto et al., 2007). A recent study also identifies Zfp105 as a NK specific transcription factor since overexpressing it promotes differentiation from hematopoietic stem cells to the NK lineage (Chambers et al., 2007).

[0132] Several genes or pathways important for T cell development genes also have functions for NK cells. For example, Gata3 and T-bet plays important roles in NK devel-

opment, maturation and homeostasis (Samson et al., 2003; Vosshenrich et al., 2006) (Townsend et al., 2004). Notch triggers initiation of T cell program, and is required to sustain or protect the cells throughout the pro-T cell stages (Maillard et al., 2005; Radtke et al., 1999; Rothenberg, 2007). Loss of Notch signalling in DN1 thymocytes convert them into dendritic cells (Feyerabend et al., 2009). Deleting of Notch in the thymus leads to accumulation of B cells in the thymus possibly by a cell-extrinsic pathway (Feyerabend et al., 2009; Radtke et al., 1999).

[0133] In contrast to its role in T cells, Notch generally suppresses NK potential in DN1 and DN2 pro-T cells until the cells progress to the committed DN3 stage (Carotta et al., 2006; De Smedt et al., 2005; Garcia-Peydro et al., 2006; Rolink et al., 2006; Schmitt et al., 2004; Taghon et al., 2007; van den Brandt et al., 2004). Nevertheless, it is proposed that transient Notch signaling is required for NK differentiation from early progenitors or stem cells (Benne et al., 2009; Haraguchi et al., 2009; Rolink et al., 2006). This may reflect the role of Notch in promoting T/NK bipotent progenitors (DeHart et al., 2005).

[0134] In the periphery, the cytokine IL-7 and the constant interaction of T cells with self peptide-MHC play a critical role in T cell maintenance (3). RT-PCR analysis indicates that many genes important for T cell commitment start to increase their expression in the transition from DN1 to DN2, with Bcl11b being the most upregulated transcription factor (4). In bony fish, Bcl11b is shown to be required for T cell precursor homing to the thymus (5). In the mouse, Bcl11b has critical roles in fetal thymocyte development and survival, and in positive selection and survival of double-positive thymocytes (6, 7).

[0135] Bcl11b is a C_2H_2 zinc finger transcription repressor (Avram et al., 2000; Cismasiu et al., 2005). Germline mutation of Bcl11b in the mouse causes thymocyte developmental block at the DN3 stage secondary to apoptosis induced by defective β-selection in thymocytes (Wakabayashi et al., 2003). Bcl11b is recently shown to be required for positive selection and survival of double-positive thymocytes (Albu et al., 2007). However, suppression of Bcl11b expression by RNA interference selectively induces apoptosis in transformed T cells but does not appear to affect normal mature T cells (Grabarczyk et al., 2007).

[0136] Here we show that the transcription factor Bcl11b was expressed in all T cell compartments, and was indispensable for T lineage development. When Bcl11b was deleted, T cells from all developmental stages acquired NK cell properties and concomitantly lost or decreased T cell-associated gene expression. These Induced T-to-Natural-Killer (ITNK) cells, which were morphologically and genetically similar to conventional NK cells, killed tumor cells in vitro and effectively prevented tumor metastasis in vivo. Therefore ITNKs may represent a new cell source for cell-based therapies.

Bcl11b is Expressed and Required in the Early T Cell Progenitors

[0137] Microarray studies indicate that expression of many genes important in T cell commitment, including Bcl11b, starts to increase in DN2 thymocytes. Among transcription factors, Bcl11b is the most drastically upregulated in the transition from DN1 to DN2 (Rothenberg, 2007). To determine Bcl11b expression in early T cells at the single cell level, we produced a lacZ knock-in allele of Bcl11b where a SA-lacZ cassette is inserted into the intron 3 to trace its expression (Song-Choon Lee, et al, unpublished). Therefore, Bcl11b expression can be traced indirectly by using Fluorescein di-3-D-galactopyranoside (FDG), a fluorescent substrate of 3-galactosidase, in flow cytometry. In hematopoietic lineages, expression of Bcl11b was only detectable in T cells (data not shown). In the thymus, almost all DN2-DN4 thymocytes expressed Bcl11b (FIG. **13***a* and FIG. **14***a*). In contrast, only about 80% of DN1 thymocytes expressed Bcl11b. Further analysis using a CD117 antibody identified that 60% of DN1a and DN1b thymocytes, which are thought to be the earliest T cell progenitors (Porritt et al., 2004), already expressed Bcl11b (FIGS. **13**B and **14***a*), suggesting a possible role of Bcl11b at the earliest T lineage specification steps.

[0138] To determine Bcl11b expression in T cells at the single cell level, we produced and analyzed a Bcl11b tdTomato knock-in mouse (FIG. **5**A-B). In hematopoietic lineages, Bcl11b was not expressed in B or myeloid cells whereas almost all DN2-DN4 and DP thymocytes, $CD4^+$ and $CD8^+$ T cells, γδ-T cells and Natural Killer T cells (NKT) expressed Bcl11b (FIG. **6**, A-C and **7**, A-C). In DN1 thymocytes, very little to no expression of Bcl11b was detected in $CD117^{++}$ cells (known as Early T-cell-lineage Progenitors (2)) (FIGS. **6**A and **7**A). During NK development, transient, low Bcl11b expression was observed in immature NK cells but not in NK precusors (NKP) or mature NK cells (FIGS. **6**D and **7**D). In contrast, the majority of thymic NK cells, identified by CD127 (8), expressed Bcl11b (FIGS. **6**D and **7**E). Moreover, in both $CD4^+$ and $CD8^+$ splenic T cells, Bcl11b transcript was reduced roughly two-fold in activated T cells ($CD44^+CD62^-L$) compared to naïve ($CD44^-CD62^+L$) cells in quantitative real time-polymerase chain reaction (qRT-PCR) analysis (FIGS. **6**E and **7**F) and exhibited a bimodal pattern of expression (FIG. **6**F).

Bcl11b Deletion Caused Loss of T Cell Identity and Acquisition of Nk-Specific Properties in T cells

[0139] The above expression and function data have demonstrated that Bcl11b is expressed in T cell precursors and required for differentiation to T cell lineage. Germline deletion of Bcl11b caused apoptosis in DN3 thymocytes in the fetal thymus but did not obviously affect DN1/2 cells (Wakabayashi et al., 2003). To further determine Bcl11b functions in T cells, we generated the conditional knockout mice ($Bcl11b^{flox/flox}$) where exon 4 was floxed (FIG. **8**A), which were crossed to the *Rosa*26Cre-ERT2 mice (9). All the thymocytes from CreERT2; $Bcl11b^{flox/flox}$ mice express Tamoxifen (OHT)-inducible Cre recombinase. Consequently, in CreERT2; $Bcl11b^{flox/flox}$ mice (PLBD line. Referred to as flox/flox in the manuscript), Bcl11b could be deleted by treating cultured cells or mice with Tamoxifen (OHT). From OHT-treated whole thymocytes from these and the control (CreERT2; $Bcl11b^{flox/+}$, referred to flox/+) mice, we sorted and subsequently cultured DN1 and DN2 cells in T cell media (Flt3 ligand and 11-7) for 2 weeks (FIG. **14***b*) on OP9-DL1 stromal cells (FIG. **8**B) (10), which support T cell development but suppress NK cell development from the progenitors (11). OP9-DL1 stromal cells express Delta-Like-1 Notch ligand and support robust T cell development (Schmitt and Zuniga-Pflucker, 2002) while normally suppressing NK cell development (Rolink et al., 2006; van den Brandt et al., 2004). All stromal cells were killed in the OHT-treated flox/flox DN1 thymocyte culture.

[0140] Flow cytometry showed that 18% of the cultured thymocytes now expressed the NK cell marker NK1.1 (DN1 in FIG. **13***c*). 24% of cells in this culture expressed NKp46,

which is primarily expressed on NK cells (FIG. **1**A) (12). These NKp46$^+$ cells did not express T cell genes CD3 or TCRβ (FIG. **8**C), and had lost both alleles of the Bcl11b exon 4 (FIG. **8**D), indicating that they did not acquire or had lost T cell features despite being co-cultured with OP9-DL1 stromal cells for 14 days. PCR genotyping of these NK1.1$^+$CD3$^-$ and NKp46$^+$CD3$^-$ cells showed that they had deleted both alleles of the Bcl11b exon 4 while those NKp46$^-$CD3$^+$ cells from the same OHT treated culture were found to still retain at least one copy of the Bcl11b cko allele. On the other hand, the control OHT-treated flox/+ and untreated flox/flox DN1 cells proliferated rapidly, and many (36%) acquired CD3 expression but not NK1.1$^+$ or NKp46$^+$ (FIG. **1**A and FIG. **8**E) consistent with Notch signalling suppressing NK development and excluding the possibility that the NKp46$^+$ cells in OHT treated DN1 cell culture were derived from NK precursor contamination (FIG. **13***c*). These data thus demonstrated that Bcl11b deficiency caused production of the NKp46$^+$ cells from DN1 thymocytes and that Bcl11b was required in early T cell development.

[0141] T cell lineage commitment is thought to occur in DN2 cells with increased expression of T cell specification genes such as Gata3, Tcf1 and Bcl11b (Ciofani and Zuniga-Pflucker, 2007; Rothenberg, 2007). Nevertheless, recent data suggest that even DN2 thymocytes still retain differentiation potentials of myeloid and NK lineages (Bell and Bhandoola, 2008). We next investigated Bcl11b function during T cell lineage commitment by deleting Bcl11b in purified DN2 thymocytes. Wild type DN2 thymocytes (−OHT) proliferated extensively on OP9-DL1 cells and gave rise to CD3$^+$ cells but no NK cells (−OHT DN2 in FIG. **13***c*). Similar to cultured DN1 thymocytes, OHT-treated flox/flox DN2 thymocytes also produced NKp46$^+$CD3$^-$ cells which killed the stromal cells, whereas control DN2 thymocytes did not (FIG. **1**A and FIG. **8**E). Similar to that in DN1 thymocyte culture, NK1.1$^+$ CD3$^-$ and NKp46$^+$CD3$^-$ cells also grew out from Bcl11b-deficient DN2 thymocytes culture on OP9-DL1 stromal cells (+OHT DN2 in FIG. **13***c*), demonstrating rapid loss of T cell differentiation potential upon Bcl11b loss in the DN2 thymocytes.

[0142] Growth of NK-like cells from Bcl11b-deficient DN1 or DN2 thymocytes appeared to be Notch signaling independent since NKp46$^+$ cells were readily produced from DN1 or DN2 thymocytes cultured on OP9 stromal cells without IL-2 (FIG. **8**F). Hence, Bcl11b has an essential function in the initial specification of the T cell lineage.

[0143] We subsequently deleted Bcl11b in DN3 thymocytes. Again, stromal cell-killing NKp46$^+$CD3$^-$ cells appeared (FIG. **1**B-C; FIG. **8**G). We purified DN3 thymoytes from OHT treated whole thymocytes from CreERT2; Bcl11b$^{flox/flox}$ and cultured them on OP9-DL1 stromal cells. Within 14 days of culturing, most of the cells became NKp46$^+$CD3$^-$ and were able to kill stromal cells. Supplement of IL-2 or IL-15 in the culture media greatly promoted proliferation and/or differentiation of these cells. Consequently most cells in the culture were NKp46' and they started to kill stromal cells within 10 days after OHT treatment (FIGS. **1**B and **1**C). NK progenitors normally do not differentiate on OP9-DL1 stromal cells. (FIG. **1**D).

[0144] The reprogramming also worked in myeloid or B cell culture media (FIG. **8**H-I), demonstrating that reprogramming to NKp46$^+$ cells was intrinsic to the Bcl11b-deficient thymocytes. To further confirm that the NKp46$^+$CD3$^-$ cells came from T cells, we purified them and examined their TCRβ locus for DNA rearrangements. These NKp46$^+$CD3$^-$ cells retained TCR β V(D)J recombination even though they no longer expressed Tcr β on the cell surface, thus genetically confirming the T cell origin of these NKp46$^+$CD3$^-$ cells (FIG. **1**D). We thus named these killer cells that were reprogrammed from T cells as Induced T-to-Natural-Killer or ITNK cells.

[0145] We next compared using microarray analysis the expression profiles of DN3 thymocytes, normal splenic NK cells that were expanded in vitro after enrichment (lymphokine-activated killer, or LAK cells, composed of >90% NK cells), and ITNKs reprogrammed from DN3 cells (FIG. **1**E). Consistent with the killing ability of ITNK cells, their expression profile was much more similar to that of LAK cells than to their parental DN3 thymocytes. Genes that showed expression difference between the parental DN3 thymocytes and their Bcl11b-deficient derivatives were listed in Table 2. qRT-PCR analysis was subsequently performed to confirm the array results (FIG. **13**F). qRT-PCR validation showed that expression of many T lineage genes, such as Notch1, Est1, Hest Gata3, Dtx1 and Tcf1 was decreased, whereas expression of genes usually associated with NK cells such as Id2 (13), IL2rβ (CD122), Zfp105 (14) and E4 bp4 (15) was upregulated (FIG. **1**F and table 1). Zbtb32 (Rog, Repressor of GATA), which is not normally expressed in DN3 cells, but plays important roles in regulating T cell activation and suppresses Gata3 activity (16), was highly expressed in ITNKs. Expression of Cdkn1c (p57KIP2), a putative direct downstream target gene of Bcl11b (17), was also drastically increased in ITNKs (FIGS. **1**F and **1**G). Indeed, p57KIP2 expression was not barely detectable in DN3 cells but drastically increased in DN3 derived iTNKs (FIGS. **1**F and **1**G). Further analysis from the array data identified 504 genes that were expressed at least two folds higher in LAKs vs DN3 thymocytes, and 366 genes in DN3 thymocyte-derived NKp46$^+$CD3$^-$ cells vs their parental DN3 thymocytes (Table 2). 70% of these 366 genes in iTNKs were found overexpressed in LAKs (FIG. **8**J). These results thus collectively demonstrated that Bcl11b was essential for maintaining the T cell expression profile and for suppressing NK cell gene expression.

[0146] We next investigated whether Bcl11b was required for T cell identity maintenance in all T cells by subjecting purified double positive (DP) thymocytes, CD4 or CD8 single positive mature T cells, to OHT treatment. These cells were then cultured on OP9-DL1 stromal cells. Similar to cultured Bcl11b-deficient DN3 thymocytes, iTNKs grew out from all T cell cultures within 10 days after Bcl11b was deleted, as demonstrated by many NKp46$^+$ cells (FIG. **15***a*, **15***b*, **15***c*). Interestingly, these iTNKs that were derived from Tcrβ-expressing T cells, still retained Tcrδ on the cell surface. In contrast to iTNKs from CD8$^+$ T cells that still expressed CD8, the CD4$^+$ single-positive T cell-derived iTNKs did not express CD4 anymore (FIG. **15***c*).

[0147] ITNKs could also be produced from mature T cells. We OHT-treated sorted double positive (DP) thymocytes, CD4$^+$ and CD8$^+$ T cells, and γδ-T cells from flox/flox mice. Many ITNKs (NKp46$^+$) were found growing in DP thymocytes and CD8$^+$ T cell cultures (FIG. **8**K-L), which effectively killed stromal cells. These ITNKs, in contrast to those reprogrammed from DN1-3 thymocytes, retained TCRβ on the cell surface. We were unable to obtain consistent production of NKp46$^+$ cells from splenic or thymic CD4$^+$ T cells, or

from γδ T cells, because these cells appeared prone to cell death in vitro once Bcl11b was deleted.

Once Bcl11b Deleted, all DN3 Thymocytes Lost T Cell Identity and Became ITNK

[0148] To estimate the reprogramming (T to NK conversion upon Bcl11b deletion) efficiency, we sorted single DN3 thymocytes from OHT-treated flox/flox thymocytes into individual wells of 96-well plates pre-seeded with OP9-DL1 stromal cells in T cell media (FIG. **9**A). Out of the 79 wells that had cells growing, 36 wells had many fast-proliferating T cells which expressed T cell surface markers including CD3 and Tcrβ (FIG. **2**A). PCR genotyping confirmed that cells in these wells did not have complete Bcl11b deletion—but deleted only one fox Bcl11b allele (FIG. **2**B, lanes T1 and T2). These cells (flox/−) nevertheless served as excellent controls for Cre toxicity because they had activated Cre recombinase. In the other 43 wells, thymocytes were reprogrammed to NKp46$^+$ stromal cell-killing ITNKs (FIG. **2**A). In these 43 wells, cells grew relatively slow but killed stromal cells. Still, from one DN3 thymocyte, up to 0.5 million of stromal-killing cells were readily obtained 14 days post OHT treatment. Flow cytometry analysis showed that almost all the cells in these wells expressed NK-specific markers NKp46 and thus were ITNKs (FIG. **2**A). IL-2 was clearly able to greatly promote proliferation of ITNKs because from one DN3 thymocyte, up to 0.5 million ITNKs were obtained with IL-2, but only about 50,000 cells without IL-2. All ITNK cells had lost both Bcl11b alleles (FIG. **2**B, lanes 11 and 12), and ITNKs of individual wells possessed unique rearranged TCRβ loci thus confirming their independent origins (FIG. **2**C). Therefore, once Bcl11b was deleted, the reprogramming efficiency of DN3 thymocytes to ITNKs could reach 100%. ITNKs from DN3 thymocytes not only expressed NK cell surface receptors and possessed similar cytotoxic functions, but were morphologically similar to LAK cells which are larger than T cells, have granules and high protein synthesis activity with abundant endoplasmic reticulum (FIG. **2**, D-E).

[0149] ITNKs were larger than thymocytes and had granules and showed evidence of high protein synthesis activity with abundant endoplasmic reticulum (FIG. **2**, D-E). Besides NK1.1 and NKp46, ITNKs expressed NKG2A/C/E, TRAIL, perforin and interferon-γ, but not some other key NK cell function genes, such as members of the Ly49 family or FasL (CD178) (FIG. **9**B-C). Similar observations were made with in vitro reprogrammed ITNK cells from DP thymocytes (table 2 and FIG. **9**D). ITNKs were unlikely to be related to thymic NK cells since they did not express CD127 (FIG. **9**E). Moreover, unlike conventional mature NK cells, most ITNKs did not express CD11b, rather, they expressed CD27, and retained killing ability even after being cultured in vitro for one month (FIG. **9**F). The iTNKs from in vitro cultured Bcl11b deficient DN3 thymocytes killed OP9-DL1 stromal cells after overnight co-culture. In fact, iTNKs retained the killing ability even cultured in vitro for at least a month. Transferring of supernatant of the iTNK cells culture to fresh stromal cells did not kill these cells, therefore cytokines secreted by iTNK cells were not sufficient, and cell-cell contact was required, for efficient killing.

[0150] We next measured the killing ability of the DN3-reprogrammed ITNKs by performing standard ^{51}Cr-release assays with three NK-sensitive cell lines: B16F10 melanoma (MHC—I low or negative) (18), RMA lymphoma, which express MHC class I molecules, and RMA-S lymphoma (TAP-1-deficient variant), which have reduced MHC class I presentation (19, 20). LAK cells generally only killed MHC-class I negative cells (FIG. **2**F). Similar to LAKs, ITNKs also selectively killed MHC—I negative B16F10 and RMA-S cells, but did not kill MHC—I positive RMA lymphoma cells (FIG. **2**F). Compared to regular LAKs, iTNKs appeared to have relatively lower killing potency. This is consistent with a lack of the full NK cell surface repertoire in the in vitro derived ITNKs (Table 2). We speculated that an in vivo microenvironment might be required for fully converting Bcl11b deficient T cells to more potent tumour cell killers.

In Vivo Reprogrammed NK Cells are More Potent Tumour Cell Killers

[0151] To exclude the possibility that ITNKs were in vitro artifacts, we deleted Bcl11b in vivo (FIG. **10**A). Two to three weeks after OHT treatment, ITNKs were detected in both the spleen (NKp46$^+$CD3$^+$) and the thymus (NKp46$^+$) from flox/flox mice but not the fox/+ controls (FIG. **3**A). Bcl11b was found deleted in these in vivo reprogrammed ITNKs (FIG. **10**B). Importantly, both CD4$^+$ and CD8$^+$ ITNKs (NKp46$^+$) were found (FIG. **10**C). Some wild type γδ-T cells expressed NKp46, however, Bcl11b deletion caused a 3-fold increase in the NKp46$^+$γδ-T cells (FIG. **3**B), which suggested that all T cell populations have reprogramming potential. The in vivo reprogrammed ITNKs could readily be expanded in NK culture conditions (FIG. **10**D), but they were not NKT cells (FIG. **10**E-F). Besides expressing NK cell-associated genes, the in vivo reprogrammed ITNKs also lost or decreased some key T cell genes such as Il7ra, Tbx21 (T-bet), Cd8 (FIG. **10**G). Consequently, TCR signaling in ITNKs appeared to be compromised (FIG. **10**H).

[0152] The in vivo analysis of ITNKs in flox/flox mice was complicated by the presence of many host T cells and NK cells (FIG. **3**A). To address this problem, and also to investigate whether in vivo reprogramming upon Bcl11b loss is cell autonomous, we transplanted 2-4 million OHT-treated DP thymocytes from flox/flox mice (CD45.2$^+$) into Rag2$^{-/-}$ Il2rg$^{-/-}$ mice (CD45.1$^+$) that lack B, T and NK cells (FIG. **11**A) (21). We chose DP thymocytes because they usually account for more than 75% of total thymocytes and could be efficiently reprogrammed in vitro to ITNKs (FIG. **8**K). Two weeks after transplantation, around 5% of splenocytes were found to be from the donor cells (CD45.2$^+$) (FIG. **3**C), and approximately 47% of them expressed NKp46 and thus were ITNKs. ITNKs lost both copies of Bcl11b and the majority of them expressed CD8 (FIG. **11**B-C). The other 53% cells (NKp46$^-$) were T cells and still retained the Bcl11b floxed allele (FIG. **11**C). The ITNKs usually accounted for 2-3% of total splenocytes. Interestingly, the majority of the splenic NKp46$^+$ ITNKs expressed CD8 (FIG. **11**B). Significant amount of NKp46$^+$ ITNKs were also present in the bone marrow and peripheral blood (FIG. **11**D). We estimated there were about 200,000 iTNK cells in the spleen alone. Nevertheless, this low ITNK number was unexpected because 2-4 millions of DP thymocytes were initially transplanted and because the T to ITNK conversion in vitro was 100%. It is possible that most of the Bcl11b-deficient DP thymocytes died either before or immediately following the conversion due to the difference between the in vivo microenvironment and in vitro culture condition, for example, the relative low levels of cytokines in the mice. No NKp46$^+$ cells were found in control mice transplanted with untreated DP thymocytes (FIG. **3**C). ITNK cells were maintained in the recipients for at

least 3 months without change in cell number, perhaps representing a dynamic balance in their numbers. Importantly the recipient mice did not show any noticeable abnormality, indicating that ITNK cells did not indiscriminately kill normal cells nor were malignantly transformed.

[0153] The in vivo iTNKs were further phenotyped by flow cytometry. Compared to those reprogrammed in vitro, the in vivo reprogrammed ITNKs appeared to express more NK surface receptors such as NKG2A/C/E and most receptors of the Ly49 family including Ly49C/I and Ly49G2 (FIG. **11**E) (table 2), and could be extensively expanded ex vivo with IL-2 or IL-15 for at least 3 weeks while still retaining their killing ability (FIG. **3**D). NK surface receptors such as Ly49 family genes including Ly49C/I, Ly49G2 were absent in the in vitro derived iTNK cells. Importantly, these iTNK cells were not NKT cells because CD1d-restricted NKT cells do not express NKp46 (Walzer et al., 2007), and the iTNKs examined in this study did not express V β 2TCR which is present in many NKT cells and recognizes non-polymorphic CD1d molecule (data not shown) (Bendelac et al., 2007).

[0154] Regular NK cells become LAKs in culture with cytokines and can be expanded for up to 7 days. After that, LAKs gradually lose proliferation and killing ability. To test the proliferation capacity of the in vivo iTNK, we cultured 2 millions splenocytes (containing approximately 50,000 iTNKs) from recipient mice in LAK condition. Most cells died in the first 3 days (FIG. **3***d*). However, within 7 days of culturing, we obtained about 2 millions $NKp46^{+}Tcr\beta^{+}$ ITNKs which accounted for 80-90% of the cell population and were able to continue proliferating for at least 3 weeks (FIG. **3***d*).

[0155] To assess functions of the in vivo iTNK cells, we used the ex vivo expanded iTNKs from the recipient mice to investigate their tumour-cell killing ability. Consistent with their expressing more killer effectors and receptors, the in vivo iTNK cells were much more potent in killing tumour cells than the regular LAKs, even after extensive ex vivo expansion These cells exhibited elevated cytotoxic potential and were also generally more potent than both in vitro ITNKs and LAKs against each of the target cells (FIG. **3**E, and FIG. **2**F). Unexpectedly, these in vivo iTNK were potent killers for all three tumour cell lines tested, regardless of their MHC—I expression status. They killed RMA cells with almost the same efficiency as killing RMA-S cells (FIG. **3**E), despite expression of some inhibitory Ly49 receptors which recognize MHC—I. Transplantable murine melanoma B16 cell lines are well-established models for studying experimental cancer therapies and NK cell tumour surveillance function (22). Injection of B16 cells into $Rag2^{-/-}Il2rg^{-/-}$ mice leads to rapid formation of metastatic foci in the lungs (23). To investigate the tumour-killing ability of the ITNK cells in vivo, we injected two million OHT-treated or -untreated DP thymocytes from flox/flox mice into $Rag2^{-/-}Il2rg^{-/-}$ recipients to allow reprogramming of thymocytes to ITNKs in vivo (FIG. **11**F). Two weeks later, each recipient was injected with 50,000 B16F10 melanoma cells. Four weeks after the initial thymocyte transplantation, recipients were sacrificed and analyzed. Mice injected with PBS or with untreated DP cells had about 200 metastatic foci in the lungs. In contrast, mice injected with OHT-treated DP thymocytes had approximately 20 tumour colonies in the lung (FIG. **3**F and FIG. **11**G). Therefore ITNKs were potent killers of tumour cells in vivo and prevented cancer progression.

Bcl11b Regulated by Notch Signalling in T Cells

[0156] Western blot indicated that in the thymocytes from CreERT2; $Bcl11b^{flox/flox}$, the Bcl11b protein levels decreased drastically 24 hours after OHT treatment. And 48 hours later, Bcl11b protein was undetectable. Hence, deletion of Bcll-lbled to rapid disappearance of Bcl11b protein (FIG. **4**A). To probe gene expression changes immediately following Bcl11b deletion in T cells, we performed expression array analysis 24 and 48 hours following OHT treatment. Microarray analysis showed that in OHT-treated flox/flox thymocytes, expression of T cell genes such as TCRβ and CD3 was already down-regulated within 24 hours (table 3). In another 24 hours, many genes associated with NK cells were expressed (table 3). Table 3 lists genes that Bcl11b loss significantly affected their expression (2 folds). Expression of several genes that are important for NK cell functions, such as NKG7, KLRD1 (CD94), PLCG and IFNG, were already increased 48 hours after OHT treatment.

[0157] Bcl11b is proposed to be regulated by Notch signaling in T cell development (24). Recent genome-wide ChIP-seq in *Drosophila* has indeed identified CG6530, the *Drosophila* orthologue of Bc111 gene, is a direct downstream target gene of Notch signalling (Krejci et al., 2009). Notch signalling normally plays an inhibitory role in NK lineage differentiation and no NK cells would grow out from bone marrow or thymocytes cultured on OP9-DL1 stromal cells. Consistent with the idea that Bcl11b acts downstream of Notch signalling in T cells, once Bcl11b was deleted, iTNK production from T cells was independent of Notch signalling because T to NK conversion occurred using either OP9 or OP9-DL1 stromal cells (data not shown).

[0158] To confirm that Bcl11b is directly regulated by Notch signalling in mouse T cells at the molecular level, we first searched within the Bcl11b gene locus for putative CSL-binding sites (CGTGGGAA) (26) at the Bcl11b locus, which were conserved between mouse and human Bcl11b genes (FIG. **4**B) (table 4). Several CSL sites were identified but we focused our attention on the ones that were conserved between mouse and human Bcl11b genes. Chromatin immunoprecipitation (ChIP) assay was subsequently performed using a CSL polyclonal antibody pulled down genomic DNA fragments from T cells. Three genomic regions were greatly enriched in the T cell samples using the CSL antibody compared to the control (FIG. **4**C). Primers flanking the putative CSL binding regions were designed to amplify the ChIP pull-down genomic DNA (FIG. **4**B). Regions 3, 4, 7 were greatly enriched in the T cell samples using the CSL antibody compared to using the antibody control (FIG. **4**C and Table 4). Region 3 is about 1.8 kb from start of the transcription. Region 4 was located 5.4 kb downstream of exon 1; and region 7 was at about 600 bp downstream of exon 2 The ChIP result thus confirmed that the canonical Notch signaling directly regulated Bcl11b in T cells (FIG. **12**).

[0159] However, it is reported that deleting CSL (RBPJk) using either CD4-Cre or Lck-Cre did not cause total T cell loss or lead to production of ITNKs (38). This discrepancy likely reflects that we acutely deleted Bcl11b in T cells for immediate functional consequences whereas if CD4-Cre is used, the deletion can occur in progenitors. Consequently, in CD4-Cre mice, the cells having defects are those from mutant progenitors and have developed mechanisms to compensate

for the loss of Bcl11b. We propose that Bcl11b is a downstream target gene of Notch signalling, and that Bcl11b, together with other Notch downstream transcription factors Gata3 and Tcf1, play pivotal roles in specification, commitment and maintenance of the T cell lineage.

[0160] We show that Bcl11b was essential for T cell development and maintenance of T cell identity. Unlike loss of Pax5 in B cells (39), however, deletion of Bcl11b did not appear to have detectable de-differentiation steps because both lymphocytes and mature T cells were readily reprogrammed to ITNKs, and ITNKs from DP thymocytes and mature T cells still retained expression of TCRβ, CD4 or CD8. This "transdifferentiation" might reflect the fact that T and NK lineages were diverted late in hematopoiesis and thus loss of one transcription factor, Bcl11b, was sufficient to cause lineage switch with 100% efficiency.

[0161] Because ITNKs reprogrammed from mature T cells retain TCRβ expression, it is possible that Bcl11b mainly functions as a suppressor of NK lineage rather than promoting and maintaining the T cell linage. Our data however do not support this possibility: ITNKs are different from NK cells, even those reprogrammed from DN1-DN2 thymocytes; Bcl11b is expressed at certain stages of NK development; Although ITNKs from mature T cells retain more T cell properties, they are still vastly different from either T cells or NK cells, and have no or diminished expression of IL7Ra, CD4, CD8, CD3 and T-bet (FIG. **10**G); Microarray data show that in OHT-treated thymocytes (Bcl11b deletion), in the first 24 hours, down-regulation of T cell-associated genes account for almost all the gene expression changes. NK-associated genes expression follows down-regulation of T cell genes and starts after 48 hours following Bcl11b deletion. Master regulators that promote a cell lineage and that are required to maintain lineage identity have been identified for several cell lineages. For example, ectopically expressing Cebpa in pro-B and pro-T cells transforms them into macrophages at a frequency of around 60% (Laiosa et al., 2006; Xie et al., 2004). 25-50% of fibroblast cells expressing MyoD convert to myogenic colonies (Davis et al., 1987). Recently, it is shown that pancreatic acinar cells expressing three TFs, Pdx1, Ngn3 and Mafa is able to convert them into insulin-expressing β cells in vivo at an estimated frequency of 20%. Additionally, loss of Pax5 in B cells enables de-differentiation of B cells to become multi-potent progenitors (Mikkola et al., 2002). Similar to Pax5 in B cells, we show here that Bcl11b is essential for T cell development and currently the only known transcription factor for T cell identity maintenance. However, unlike de-differentiation in B cell upon loss of Pax5 (Cobaleda et al., 2007), deletion of Bcl11b in T cells does not appear to have obvious or prolonged de-differentiation steps because both pro-T and mature T cells readily convert to ITNKs. Moreover, ITNKs from DP thymocytes and mature T cells still retained Tcrβ expression. This may reflect the fact that T and NK lineages are diverted late during T cell development in the thymus and thus loss of one transcription factor, Bcl11b, is sufficient to convert T cells into iTNK cells with 100% efficiency. Our study therefore adds Bcl11b to the collection of transcription factors that play pivotal roles in hematopoietic lineage specification, commitment and maintenance.

[0162] NK cell-based therapies hold promise in cancer treatment. We are now able to reprogramme T cells to ITNKs, which can be extensively expanded but are not malignantly transformed. Rather, they effectively killed tumour cells in vitro and eliminated metastatic cells in mice but did not appear to attack normal cells. Therefore, ITNK cells may serve as a new cell source for cancer immunotherapy and other cell-based therapies.

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FIGURE LEGENDS

[0245] FIG. **1**. Bcl11b is essential for T cell development and for maintaining T cell identity. Thymocytes from flox/flox or flox/+ control mice were treated, or not, with OHT then sorted into DN1 or DN2 subsets, and cultured on OP9-DL1 stromal cells. (A) Flow cytometry profiles of cultured DN1 and DN2 thymocytes (+OHT) in the absence of IL-2. Numbers refer to percentage of cells in the gate. Data are representative of three experiments. (B) Flow cytometry profiles of cultured flox/flox DN3 thymocytes (±OHT) supplemented with IL-2. Data are representative of three experiments. Bcl11b-deficient DN3 thymocytes lost T cell identity and converted to NKp46 expressing cells. −OHT: non-treated cells; +OHT: treated cells. (C) Killing of OP9-DLI stromal cells by OHT-treated flox/flox DN3 thymocytes. Scale bar: 40 μm. The NKp46$^+$ cells from Bcl11b deficient DN3 thymocytes (+OHT) killed OP9-DL1 stromal cells effectively. (D) DNA from purified NKp46$^+$ cells was prepared and subjected to PCR to detect DJ (top) and VDJ (bottom) recombination at the TCRβ locus. T: T cells growing from untreated DN3 thymocytes; N1 and N2: sorted NKp46$^+$ cells growing from OHT-treated flox/flox DN3 thymocytes; Thy: wild type whole thymocytes; B: B cells; GL: germline band; H_2O: no DNA template in PCR. Numbers indicate DJ recombination products. The NKp46$^+$ cells from Bcl11b deficient DN3 thymocytes still retained V(D)J recombination at the Tcrβ locus even though they did not express Tcrβ. (E-G) Microarray analysis of gene expression in NKp46$^+$CD3$^+$ ITNK cells from DN3 thymocytes (I1-I4), IL-2-expanded NK cells (LAK; L1-L4) and sorted DN3 flox/flox thymocytes (DN3; D1-D4) were subjected to expression. (E) Two-way hierarchical cluster map of the array data. Column numbers (I1-I4 for instance) refer to 4 independent RNA samples for each cell type and rows represent individual transcripts. Scale indicates the log2 value of normalized signal level. Comparison of expression profiles of parental DN3 thymocytes, iTNK cells derived from DN3 thymocytes and regular NK cells (LAKs). RNA samples were made from 4 mice for each cell type. (F) qRT-PCR validation of gene expression of selected genes among ITNKs, LAKs and DN3 cells. Bars are mean+SD of 3 samples. In each histogram in FIG. **1** (F), the first bar represents DN3 cells, the second bar represents ITNKs and the third bar represents LAKs. (G) qRT-PCR validation of gene expression difference among DN3, iTNK and LAK cells. Expression of T cell specific genes was generally decreased, and expression of NK-specific genes was greatly

increased in the NK-like cells. Zbtb23 (Rog) and Cdkn1c (p57Kip) were not normally expressed in DN3 thymocytes. In each histogram in FIG. **1** (G), the first bar represents LAK cells, the second bar represents ITNKs and the third bar represents DN3 cells.

[0246] FIG. **2**. Efficient reprogramming of T cells to ITNKs. (A) Representative flow cytometry profiles of ITNKs reprogrammed from single flox/flox DN3 cells. Numbers refer to percentage in total cells. T: T cells that did not have complete Bcl11b deletion. Data are representative of three experiments. NKp46$^+$ iTNKs derived from single Bcl11b-deficient DN3 thymocytes in individual wells (96-well plate) co-cultured with OP9-DL1 stromal cells. T: cells that expressed T cell genes and Bcl11b was not completely deleted; iTNK: cells that had deleted both copies of Bcl11b and expressed NKp46.

[0247] (B) PCR genotyping of Bcl11b deletion in two representative T cell (T1, T2) and ITNK (I1, I2) wells. flox: floxed allele; del; deletion allele. −OHT: no OHT treatment; H_2O: no template control. PCR-genotyping indicated that cells in some wells did not have complete Cre-loxP recombination (T1 and T2). These cells had one deletion allele and one cko allele at the Bcl11b locus. On the other hand, all the NKp46$^+$ cells had Bcl11b completely deleted (11 and 12). No deletion was detected in cells without OHT treatment (−OHT).

[0248] (C) DJ recombination at the TCRβ locus of five ITNK wells (I1-I5) showing unique DJ recombination. L: DNA ladder; Thy: wild type thymocytes. (D) Giemsa stain of parental DN3 thymocytes (T) and ITNK cells. Scale bar: 20 μm. (E) Transmission electron micrograph of an ITNK cell. 1: Nucleus; 2. Golgi body; 3. Granule; 4. ER. Scale bar: 2 μm. (e) Electron Transmission Microscopy image of ITNK cells shows prominent Golgi and ERs, and granules. Arrows: 1=nucleus; 2=ER; 3=granule; 4=golgi. (F) Cytotoxicity of ITNKs (labeled as "+OHT") and LAKs measured in standard ^{51}Cr release assays with B16F10, RMA and RMA-S tumor cell targets at the indicated effector-to-target (E:T) ratios. −OHT: flox/flox T cells. Data are mean of triplicate wells. In vitro derived ITNK cells from DN3 thymocytes killed tumour cells effectively. Both LAK and ITNK cells killed MHC—I negative B16F10 melanoma and RMA-S lymphoma cells.

[0249] FIG. **3**. ITNKs reprogrammed in vivo were potent tumour cell killers. (A) Flow cytometric analysis of thymocytes and splenocytes from OHT treated flox/flox and flox/+ mice. Numbers refer to percentage in lymphocyte gate. Data are representative of four mice. (B) Analysis of ITNKs from thymic γδ cells in OHT treated flox/flox mice. Data are representative of two mice. (C) ITNKs production in Rag2$^{-/-}$ Il2rg$^{-/-}$ recipients injected with flox/flox DP thymocytes. Two weeks after injection, donor (CD45.2$^+$) and host (CD45.1$^+$) splenocytes were analyzed. Numbers refer to percentage of lymphocyte gate. Plots are representative of 15 mice from three independent experiments. Donor cells were identified by CD45.2 staining. About 5% of splenocytes were donor derived and roughly half of these donor-derived cells were NKp46$^+$ iTNKs. (D) Ex vivo expansion of ITNKs in IL-2 from splenocytes of the recipient mice. Viable cells were counted and analyzed (bottom panel) at the indicated time points. Numbers refer to percentages. Most cells in the culture were ITNKs because they expressed NKp46, TCRβ, NK1.1 and NKG2D. Bars are mean±SD of 4 samples. Data are representative of three experiments. (d) Ex vivo expansion of in vivo reprogrammed iTNK cells starting from splenotypes of four Rag2$^{-/-}$Il2γc$^{-/-}$ recipient mice. These cells were able to proliferate extensively in the culture for up to 3-4 weeks. Bottom panel: iTNK cells (NK1.1$^+$ and/or NKp46$^+$) accounted for the majority of the cells in the culture after one week culturing. (E) The ex vivo-expanded ITNKs (labeled as "+OHT") were used in ^{51}Cr release killing assays with B16F10, RMA and RMA-S tumor cell targets at the indicated effector-to-target (E:T) ratios. −OHT: flox/flox T cells. Data are mean of triplicate wells. Results are representative of three experiments. Ex vivo expanded iTNKs were more potent killers for tumour cells than LAKs. iTNKs effectively killed tumour cells of either MHC—I positive or negative.

[0250] (F) ITNKs prevented tumour metastasis. Rag2$^{-/-}$ Il2rg$^{-/-}$ recipients first transplanted with treated (+OHT) or untreated (−OHT) flox/flox DP thymocytes or PBS. Recipients were subsequently injected intravenously with 50,000 B16F10 melanoma cells. Lung tumour colonies were enumerated two weeks after tumour challenge. Data are from individual mice and bar represents the mean. (G) In vivo iTNKs effectively eliminated B16F10 melanoma cells in mice. Many metastatic colonies were visible in the lung of the control mice that were injected with either PBS (no cells) or untreated DP thymocytes (−OHT). Very few metastatic colonies existed if OHT-treated DP thymocytes were injected and hence iTNK were produced (+OHT).

[0251] FIG. **4**. Bcl11b is a direct downstream target gene of Notch signaling. (A). Bcl11b protein in T cells following OHT treatment detected by Western blot. (B) Schematic of the Bcl11b locus showing putative CSL binding sites (BS) and that of an irrelevant control binding site (CTL). (C) Genomic DNA was prepared from immunoprecipitation of thymocytes, using CSL or control IgG antibodies, and was amplified using primers flanking the putative CSL or the control binding sites at the Bcl11b locus. Three Bcl11b binding regions: Region 1, about 1.8 kb from start of the transcription; Region 2, 5.4 kb downstream of exon 1; region 3, about 600 bp downstream of exon 2. CSL: CSL antibody; IgG: control IgG. Fold-enrichment was calculated relative to the IgG control (set to 1). Bars are mean±SD of triplicate. In the histogram in FIG. **4** (*c*), the first bar represents CSL and the second bar represents IgG.

[0252] FIG. **5**. Generation of the Bcl11b-tdTomato reporter mouse. (A) The tdTomato cassette was targeted to the 3' UTR of the Bcl11b locus. (B) Insertion of the tdTomato cassette at the Bcl11b 3' UTR did not affect T cell development. Numbers refer to percentage of lymphocytes gate. Data are representative of three mice.

[0253] FIG. **6**. Detection of Bcl11b expression in hematopoietic lineages using the Bcl11b-tdTomato reporter mice. Leukocytes from the thymus, spleen and bone marrow of Bcl11b$^{fd/+}$ mice were labeled with antibodies for flow cytometric analysis. Bcl11b-expressing cells had red fluorescence. Solid line refers to Bcl11b$^{fd/+}$ mice and dashed line refers to wild type mouse. (A) CD4$^-$CD8$^-$ double negative (DN; DN1-DN4) thymocyte subsets. DN1: CD44$^+$CD25$^-$; DN2: CD44$^+$CD25$^+$; DN3: CD44$^-$CD25$^+$; DN4: CD44$^-$CD25$^-$. (B) Double positive (DP) thymocytes (CD4$^+$CD8$^+$), splenic CD4$^+$ and CD8$^+$ T cells, thymic γδ T cells, and splenic NKT cells (CD3$^+$CD1d$^+$). (C) Bone marrow B cells (CD19$^+$ B220$^+$) and myeloid cells (CD11b$^+$Gr-1$^+$). (D) Splenic (CD3$^-$), and thymic (CD3$^-$CD4$^-$CD8$^-$) NK cells. NKP: NK cell precursor; Immature: NK1.1$^+$CD27$^+$CD11b$^-$ and NK1.1$^+$CD27$^+$CD11b$^+$. (E) qRT-PCR of Bcl11b expression in sorted splenic naïve (CD44CD62$^-$L$^+$) and activated (CD44$^+$

CD62L$^-$) T cells population. Bcl11b expression was calculated relative to that in CD8$^+$CD44$^+$CD62L$^-$ (set to 1). Bars are mean±SEM of 3 samples. (F) Quantification of Bcl11b expression in naïve and activated T cells in the Bcl11b$^{fd/+}$ mice. Percentages refer to the indicated T cell subsets in Bcl11b$^{fd/+}$ mice. All FACS data in this figure are representative of three experiments.

[0254] FIG. **7**. Strategies for identification of cell populations for flow sorting and analysis. (A) Identification of double negative (DN) thymocyte (DN1-DN4) populations defined by Lin$^-$ and expression of CD25 and CD44. DN1 subpopulations were defined by expression of CD117 (c-Kit). Numbers refer to percentages. (B) Identification of γδ T cells. (C) Identification of NKT cells in the spleen by first gating (or, prior to FACS sorting, magnetically depleting) out B cells. INKTs were CD3$^+$ and stained positively by CD1d dimer. (D) Identification of NK precursors (CD3$^-$CD122$^+$ NK1.1$^-$) and NK cell subsets (NK1.1$^+$CD27$^+$CD11 b$^-$, NK1.1$^+$CD27$^+$CD11b$^+$, NK1.1$^+$CD27$^-$CD11b$^+$) cells. (E) Thymic NK cells were defined as NK1.1$^+$CD127$^+$ thymocytes. (F) Identification of naïve (CD44$^-$CD62L$^+$) and activated (CD44$^+$CD62L$^-$) T cells.

[0255] FIG. **8**. In vitro analysis of Bcl11b-deficient T cells. (A) Schematic diagram of the Bcl11b conditional knockout allele. Bcl11b exon 4 was flanked by loxP sites. Indicated DNA fragments were detected by the 5' probe in Southern blot analysis of targeted ES cells. Southern blot analysis of the targeted ES cell clones using a 5' probe which detected a 27 kb wild type BamHI band. The same probe hybridized to a 12.6 kb fragment in the conditional knockout clones (cko/+) and a 17.5 kb fragment in clones that did not have the 5' IoxP site (+/−). (B) Experimental design for the analysis of Bcl11b-deficient DN thymocytes. Whole thymocytes from CreERT2; Bcl11b$^{flox/flox}$ (fox/fox) or CreERT2; Bcl11b$^{flox/+}$ (flox/+) mice were treated with OHT (+OHT) or left untreated (−OHT) for 48 hr then sorted into the indicated subset and cultured on OP9-DL1 stromal cells for 2 weeks. (C) NKp46$^+$ CD3$^-$ cells from DN1 and DN20HT-treated flox/flox thymocytes did not express TCRβ. Numbers refer to percentage of cells. Data are representative of two experiments. (D) Homozygous Bcl11b deletion in ITNK (NKp46$^+$CD3$^-$) but not in T (NKp46$^-$CD3$^+$) cell populations from DN1 and DN2 cultures. flox: conditional knockout allele; del: deletion allele. H_2O: no DNA template control. (E) No NKp46$^+$ cells but T cells were obtained from untreated flox/flox thymocytes. (F) NKp46$^+$TCRβ$^-$ cells from OHT-treated DN1 and DN2 flox/flox thymocytes in the absence of IL-2 or IL-15 cultured on OP9 stromal cells. (G) NKp46$^+$TCRβ$^-$ cells were detected in OHT-treated DN3 flox/flox, but not flox/+, thymocytes in T cell media. (H) Reprogramming of Bcl11b-deficient DN3 thymocytes to NKp46$^+$ cells in myeloid cell culture condition. (I) Reprogramming of Bcl11b-deficient DN3 thymocytes to NKp46$^+$CD19$^-$ cells in B cell culture condition. (J) Venn diagram comparison of the upregulated (>2-fold) genes between LAK vs DN3 (green) and ITNK vs DN3 (purple) shows a significant overlapping between the two gene lists. (K) ITNKs from DP flox/flox thymocytes treated with OHT and cultured on OP9-DL1 in the presence of IL-2. Untreated cells died rapidly under this condition. (L) ITNKs from splenic flox/flox CD8$^+$ T cells treated with OHT cultured on OP9-DL1 in the presence of IL-2. All FACS data in this figure are representative of 2-4 experiments.

[0256] FIG. **9**. Characterization of in vitro reprogrammed ITNK phenotype. (A) Experimental design for reprogramming of single DN3 thymocytes to ITNK. Whole thymocytes from flox/flox mice were treated with OHT (+OHT) or left untreated (−OHT) and 48-hours later single DN3 cells were sorted and seeded on OP9-DL1 stromal cells in 96-well plates for 10-14 days supplemented with IL-2. (a) Experimental design for analyzing single DN3 thymocytes conversion to iTNKs. DN3 thymocytes (either treated with OHT, or untreated) were sorted into individual wells of 96-well plates pre-seeded with OP9-DL1 stromal cells. Two weeks (with 112) or three weeks (without 112) later, the OHT-treated DN3 cells (Bcl11b-deficient) converted to iTNKs, confirmed by FACS analysis and genomic DNA PCR. (B-C) Expression of intracellular (TRAIL, perforin, IFNγ) and NK cell surface markers by the reprogrammed ITNK from DN3 thymocytes in vitro. (D) Expression of NK cell markers by ITNKs reprogrammed from Bcl11b-deficient DP thymocytes in vitro. (E) ITNKs did not express CD127 and thus were not thymic NK cells. (F) Analysis of CD27 and CD11b in bulk-cultured ITNKs reprogrammed from DN3 thymocytes. All FACS data are representative of three experiments.

[0257] FIG. **10**. Analysis of in vivo reprogrammed ITNK cells in the flox/flox mouse. (A) Experimental design for the analysis of in vivo reprogrammed ITNK cells. flox/flox or flox/+ mice were treated with Tamoxifen by oral gavage on three consecutive days, and the thymi and spleens were analyzed 2-3 weeks later. We observed a 5-10 fold reduction in total thymocytes and about 2-fold reduction in splenocytes in the treated flox/flox mice compared to treated flox/+ control mice. (B) PCR of Bcl11b deletion in ITNK (NKp46$^+$CD3$^+$ and NKp46$^+$CD3$^-$) cell populations in flox/flox mice. flox: conditional knockout allele; del: deletion allele. H_2O: no DNA template control. All the NKp46$^+$CD3$^+$ and NKp46$^+$ CD3$^-$ cells in the thymus were ITNKs. Analyzing ITNKs in the spleen was more complicated due to the presence of many NKp46$^+$ conventional NK cells. However, most of the NKp46$^+$CD3$^+$ cells in the spleen had Bcl11b deficiency and thus were ITNKs. PCR data are representative of three experiments. (C) Flow cytometric analysis of CD4 and CD8 expression in NKp46$^+$ ITNKs. Note that both CD4 and CD8 expression was down in ITNKs (CD4$^+$NKp46$^+$ or CD8$^+$NKp46$^+$) compared to CD4$^+$NKp46$^-$ or CD8$^+$NKp46$^-$ T cells. (D) Flow cytometric analysis of cells following ex vivo expansion of whole thymocytes or splenocytes from OHT treated mice. (E) Flow cytometric analysis of CD1d-restriced NKT cells in thymus and spleen. Total lymphocytes and CD19$^-$ splenocytes were gated in the thymus and spleen, respectively. Note the reduction of NKT cells in the OHT-treated flox/flox mice. (F) Analysis of CD1d-restricted cells in the ex vivo-expanded ITNK culture. Numbers refer to percentages in lymphocyte gate. All FACS data in this figure are representative of 3-4 individual mice. (G) qRT-PCR analysis of several key T or NK cell-associated genes in CD8$^+$ T cells, CD8$^+$ ITNKs and LAKs. Bars are mean±SEM of 3 samples. The highest expression level for each gene was chosen as 1. (H) Splenocytes from flox/flox or flox/+ mice treated with Tamoxifen were stained with NKp46, NK1.1, CD8 and CD3 to confirm expression of CD3 on ITNKs. A separate aliquot was loaded with Indo-1, stained with antibodies to NKp46, NK1.1 and CD8 and analyzed for calcium flux by flow cytometry. Top panel: Phenotype of splenocytes from flox/flox or flox/+ mice indicating gated T cells (CD3$^+$NKp46$^-$) and ITNKs (CD3$^+$ NKp46$^+$) cells. Numbers refer to percentages in gates of total lymphocytes. Lower panel: Calcium flux plots from the indicated cell subset. A baseline was established at the start of the

assay, before acquisition was interrupted and anti-CD3 (145-2C11) was added (first arrow). CD3 was then cross-linked by addition of anti-hamster secondary antibody (second arrow). Ionomycin was added (third arrow) as a positive control. Numbers in gates refer to responders (upper gate) and non-responders (lower gates) after addition of anti-hamster antibody. Data below calcium plots show ratio of responders to non-responders. Data are representative of two mice.

[0258] FIG. **11**. In vivo reprogrammed ITNKs from DP thymocytes prevented tumour metastasis. (A) Experimental design for the analysis of in vivo reprogramming of DP thymocytes to ITNKs. Whole thymocytes from flox/flox mice were treated with OHT (+OHT) or left untreated (−OHT) and 48-hours later DP cells were sorted and injected intravenously into $Rag2^{-/-}Il2rg^{-/-}$ mice. Two weeks later, splenocytes, bone marrow (BM) and peripheral blood cells (PB) were analyzed by flow cytometry fro ITNKs. (B) Most ITNKs in the spleen were $CD8^{+}$. Numbers in gates refer to percentages. Data are representative of three experiments. (C) ITNKs had complete Bcl11b deletion whereas donor derived NKp46 cells still retained at least one copy of the foxed allele. PCR data are representative of two individual experiments. (D) ITNKs were also found in bone marrow and peripheral blood. About 1.0% of bone marrow and 6-7% of peripheral white blood cells expressed NKp46 and thus ITNKs in the recipients injected with Bcl11b-deficient DP thymocytes. (E) Expression of additional NK cell surface markers on the in vivo reprogrammed ITNKs. The in vivo iTNKs expressed more NK-specific receptors such as Ly49C/I and Ly49G2. (F) ITNKs prevented tumour metastasis. $Rag2^{-/-}Il2rg^{-/-}$ recipients were transplanted with treated (+OHT) or untreated (−OHT) flox/flox DP thymocytes or PBS. Recipients were subsequently injected intravenously with 5×10^{4} B16F10 melanoma cells. Lung tumour colonies were enumerated two weeks after tumour challenge. Experiment was performed twice. (G) Plot shows inverse correlation between the percentage of ITNK cells (squares) obtained from recipient mice following in vivo reprogramming and tumor challenge and the number of lung colonies (circles) observed. Data are individual mice and are representative of two independent experiments, each with 5 mice per group. Chart shows that in vivo the percentages of ITNKs in spleen (squares) correlated with reduction of metastatic sites (+OHT circles) in the $Rag2^{-/-}Il2\gamma c^{-/-}$ mice after injection of OHT treated DP thymocytes. The −OHT squares and circles represent iTNKs and the metastatic sites respectively in recipient mice that were injected OHT untreated DP thymocytes. In mice injected with OHT-treated DP thymocytes, about 4% of splenocytes were iTNKs. FIG. **12**. A working model showing that Bcl11b acts downstream of Notch signaling and promotes T cell development and maintains T cell identity.

[0259] FIG. **13**. Bcl11b is expressed in early T cell precursors and is essential for T cell differentiation.

[0260] a. Expression of Bcl11b in thymocytes from Bcl11b-lacZ knock-in mice using the fluorescent substrate FDG. Almost all of the DN2-DN4 thymocytes were stained positively for FDG. However a significant DN1 population did not express Bcl11b.

[0261] b. Detection of Bcl11b expression in the five DN1 subpopulations. Approximately half of the DN1a and DN1b thymocytes, which were $CD117^{+}$ and were thought to contain the true T cell progenitors, expressed Bcl11b.

[0262] c. Top, acute loss of Bcl11b caused DN1 thymocytes to express NK-specific genes NK1.1 and NKp46 on OP9-DL1 stromal cells. Bottom, deleting Bcl11b in DN2 thymocytes gave rise to the same phenotype of losing T cell differentiation potential and converting to NK-like cells.

[0263] FIG. **14**.

[0264] a. Left panel: different double negative (DN) thymocyte populations defined by expression of CD25 and CD44. Right panel: five subpopulations of DN1 thymcoytes based on expression of CD24 and CD117 (c-Kit).

[0265] b. Flow chart of analyzing Bcl11b-deficient DN1 thymocytes. The Bcl11b-deficient cells (+OHT) acquired NK properties while the untreated ones (−OHT) proliferated and differentiated into T cells on OP9-DL1 stromal cells.

[0266] FIG. **15**.

[0267] a. Double positive (DP) thymocytes expressed NKp46 after Bcl11b deletion. The untreated DP cells died in T cell media about one week after plated on OP9-DL1 stromal cells (not shown).

[0268] b. Purified CD8 single positive cells (−OHT) proliferated on OP9-DL1 stromal cells. They did not express NKp46. Once Bcl11b was deleted, 38% of the cells now expressed NKp46 which killed the stromal cells. Note that these iTNKs still expressed Tcrβ and CD8.

[0269] c. Purified CD4 single positive cells (−OHT) growing in T cell media (left). Bcl11b deletion (+OHT) caused these CD4 T cells to express NKp46. Note that most of the cells now did not express CD4 anymore.

Materials and Methods

[0270] 1.1.1 Mice

[0271] The Bcl11b conditional knockout targeting vector was constructed using recombineering (Liu et al., 2003), and the mice ($Bcl11b^{flox/flox}$) were made according to a standard gene targeting approach in ES cells. The $Bcl11b^{flox/flox}$ mice were crossed to Cre-ERT2 mice to generate Cre-ERT2; $Bcl11b^{flox/flox}$ mice. Cre-ERT2; mice were a mixed C57BL/6J and 129S5 genetic background. A SA-lacZ cassette was targeted into the intron 3 of Bcl11b gene in Bcl11b-lacZ reporter mice (Song-Choon Lee and Pentao Liu, unpublished). All mice were $NK1.1^{+}$ by flow cytometry, suggesting that they had inherited the C57BL/6 haplotype at the NK gene complex. Bcl11b tdTomato reporter mice were constructed by inserting the tdTomato cassette into the 3' UTR of Bcl11b. Bcl11b tdTomato mice are on a C57BL/6 background. $Rag2^{-/-}Il2rg^{-/-}$ are on a C57BL/6 background. Both C57BL/6 and 129S5 have the $H\text{-}2^{b}$ haplotype at the MHC. All animal experiments were performed in accordance with the UK 1986 Animals Scientific Procedure Act and local institute ethics committee regulations.

[0272] 1.1.2 Reprogramming of T Cells to ITNKS In Vivo Flox/Flox

[0273] To test for the in vivo reprogramming of endogenous T cells to ITNK, Cre-ERT2; $Bcl11b^{flox/flox}$ and Cre-ERT2; $Bcl11b^{flox/+}$ mice were given 3 doses of 1 mg Tamoxifen (indicated in the text as OHT) (Sigma) dissolved in sunflower oil by oral gavage on 3 consecutive days. Mice were analysed 2-3 weeks later. For the in vivo reprogramming of in vitro-treated thymocytes, thymocytes from Cre-ERT2; were treated with 4-hydroxytamoxifen (indicated in the text as OHT) (Sigma) or left untreated for 48 hours. $2\text{–}4\times10^{6}$ DP

thymocytes were then sorted and injected intravenously into $Rag2^{-/-}Il2rg^{-/-}$ recipient mice without irradiation. At various time points thereafter, blood, bone marrow and/or splenocytes were prepared for analysis.

[0274] 1.1.3 PCR Genotyping and qRT-PCR

[0275] To extract genomic DNA, sorted cells were incubated in 400 μl of lysis buffer (50 mM Tris with pH 8.0, 100 mM NaCl, 25 mM EDTA with pH 8.0, 0.5% SDS, and 0.5 mg/ml Proteinase K) at 65° C. for 2 hrs. Genomic DNA was precipitated by adding 500 μl of isopropanol into cell lysis buffer. After centrifugation, DNA was washed once with 500 μl 70% ethanol and air dried before being re-suspended as template for PCR. The Bcl11b cko allele and the deletion after Cre-loxP recombination were detected by PCR with primers described in Table 4. PCR primers to detect TCRβ D-J and V-DJ are also listed in Table 4. For qRT-PCR, RNA was isolated using the RNAqueous Micro Kit (Ambion) from FACS sorted cells. After DNase I treatment, RNA was reverse transcribed to make cDNA with Super Script 11 (Invitrogen). qRT-PCR was performed with either SYBR (Invitrogen) or Taqman Master Mix (ABgene). cDNA input was standardized and PCR was performed for 40 cycles. Primers for qRT-PCR are listed in Table 4.

[0276] FDG Staining

[0277] For FDG staining, cells were first surface stained as above. Cells were then warmed at for 5 minutes before 20 μl pre-warmed FDG (Sigma) was added for a further 1 minute. The reaction was quenched by addition of 2.0 ml ice-cold PBS plus 1% BSA, and the cells were incubated on ice for a further 30 minutes. The cells were centrifuged and resuspended in PBS before analysis.

[0278] 1.1.4 Flow Cytometry and Cell Sorting

[0279] Cells from spleen, thymus and bone marrow were mechanically disrupted and the red blood cells were removed with ACK lysis buffer (Lonza). Blood was collected into EDTA tubes (Sarstedt). In vitro-cultured cells were collected and washed with PBS/1% BSA prior to antibody labelling. For all cells, Fc receptors were blocked with anti-CD16 (2.4G2) prior to antibody labelling. Antibodies to the following antigens were used: CD3s (145-2C11), CD4 (L3T4), CD8a (53-6.7), CD25 (PC61), CD44 (IM7), CD122 (TM-131), CD27 (LG.3A10), CD11b (M1/70), CD45.2 (104), TCRβ (H57-597), CD117 (2B8), NK1.1 (PK136), CD49b (DX5), NKp46 (29A1.4), Ly49C/I (5E6), Ly49G2 (4D11), Ly49D (4E5). All antibodies were from BD Biosciences or eBioscience. Cells were incubated with antibody for 30 minutes at 4° C. before being washed. In some cases biotinylated antibodies were revealed by incubation with fluorochrome-conjugated streptavidin for a further 20 minutes at 4° C. CD1d-restricted NKT were detected by labelling cells with CD1d-mouse IgGl Fc fusion protein (BD Biosciences) loaded with α-galactosylceramide (Kirin), followed by fluorochrome-conjugated anti-mouse IgGl (BD Biosciences). Data acquisition was performed using a FACSCalibur (BD Biosciences), LSR II (BD Biosciences) or a FC 500 (Beckman Coulter) with dead cells excluded based on scatter profile or DAPI inclusion. Analysis was performed using FlowJo (Tree Star) software. Sorting was performed using a MoFlo (DAKO) or FACS Aria (BD Biosciences).

[0280] 1.1.5 OP9 Stromal Cell Culture

[0281] OP9 stromal cells were cultured in alpha-MEM (Sigma) with 10% FCS (heat inactivated at 56° C. for 30 min), 1% penicillin/streptomycin, and 2 mM L-glutamine (Life Technologies). OP9-DL1 stromal cells were cultured in alpha-MEM (Sigma) with 20% FCS, 1% penicillin/streptomycin, and 2 mM L-glutamine (Life Technologies). Cells were passaged every 2 to 3 days by trypsinization (Invitrogen). A monolayer (70%-80% confluent) of OP9 or OP9-DL1 cells was prepared 24 hours prior to co-culture.

[0282] 1.1.6 OHT Treatment In Vitro

[0283] Thymocytes or splenocytes from Cre-ERT2; $Bcl11b^{flox/flox}$ mice were cultured in T cell medium with 1 μM 4-hydroxytamoxifen (indicated in the text as OHT) at 37° C. for 48 hrs. After this time, cells were washed and resuspended with fresh media. T cell media: RPMI-1640, 10% FCS, 1% penicillin/streptomycin, 2 mM L-glutamine, 5 ng/ml muFlt-3L, 5 ng/ml hulL-7. All cytokines used in this study were purchased from PeproTech.

[0284] 1.1.7 Reprogramming of T Cells to ITNKS In Vitro

[0285] After OHT treatment, thymocytes were sorted by FACS and co-cultured with OP9-DL1 in T cell culture media (3,000 cells per well in 24-well plates). To promote ITNK proliferation, 20 ng/ml mulL-15 or 100 ng/ml hulL-2 was supplemented in T cell medium as indicated. Every three days, half of the media was replaced with fresh T cell media with IL-15 or IL-2 as indicated in text. Every seven days, cells were collected by vigorous pipetting, filtered through cell strainers and transferred to new tissue culture plates pre-seeded with fresh OP9-DL1 stromal cells. Fourteen days after OHT treatment, cells were collected and analyzed by FACS. For analysis of ITNK production in myeloid cell differentiation conditions, IMDM was used supplemented with 10% FCS, 1% penicillin/streptomycin, 2 mM L-gluatamine, 1 ng/ml hulL-7, 5 ng/ml muFlt-3L, 10 ng/ml hulL-3, hulL-6, stem cell factor (muSCF), and granulocyte/macrophage colony-stimulating factor (muGM-CSF). Cells were cultured on OP9 stromal cells. For analysis of ITNK production in B cell differentiation conditions, IMDM was used supplemented with 10% FCS, 1% penicillin/streptomycin, 2 mM L-gluatamine, 5 ng/ml hulL-7, 5 ng/ml muFlt-3L. Cells were cultured on OP9 stromal cells.

[0286] 1.1.8 Reprogramming of Single Thymocyte to ITNKs

[0287] Thymocytes of Cre-ERT2; $Bcl11b^{flox/flox}$ were treated with OHT as above. Single DN3 thymocytes were sorted directly into individual wells of a 96-well plate pre-seeded with OP9-DL1 stromal cells in T cell medium supplemented with 100 ng/ml hulL-2. Medium was changed every three days. After 10-14 days cells were analyzed in flow cytometry. Genomic DNA was extracted for genotyping of the Bcl11b locus and for amplifying 6TCR rearrangement with PCR.

[0288] 1.1.9 Tumour Cell Killing Assay

[0289] B16F10 melanoma ($H\text{-}2^b$), RMA lymphoma and RMA-S lymphoma ($N\text{-}2^b$ TAP-1-deficient variant) were maintained in RPMI-1640, 5% FCS, 1% penicillin/streptomycin, 2 mM L-glutamine. For killing assays, target cells were washed and incubated with 0.1 μCi $Na_2{}^{51}CrO_4$ (Perkin Elmer) for 45 mins. at 37° C. The cells were then washed and added in triplicate to effector cells at the indicated E:T ratio. Plates were incubated for 4 hours at 37° C. before the supernatant was tested for chromium release in a scintillation counter. Percent specific lysis was calculated as (experimental release—spontaneous release)/(maximum release—spontaneous release)×100.

[0290] T Cells to iTNKs In Vivo

[0291] Thymocytes from Cre-ERT2; $b^{flox/flox}$ were treated with OHT as above. $2\text{–}4\times10^6$ DP thymocytes were sorted and

injected intravenously into $Rag2^{-/-}Il2\gamma c^{-/-}$ recipient mice without irradiation. At various time points thereafter, blood and/or splenocytes were prepared for analysis.

[0292] 1.1.10 ITNK Ex Vivo Expansion and LAK Culturing

[0293] For ex vivo expansion, splenic ITNK cells were enriched using the NK Isolation Kit (Miltenyi) and cultured for 6-9 days at 1×10^6 cells/ml in RPMI 1640 medium containing 10% FCS/50 μM 2-mercaptoethanol/2.0 mM L-glutamine and 1000 U/ml hIL-2 (Chiron). The cells were split every two days and supplemented with fresh IL-2. Purity was always >90%. For culturing reprogrammed ITNK cells ex vivo, whole splenocytes were cultured without pre-enrichment.

[0294] 1.1.11 Tumour Experiments In Vivo

[0295] After OHT treatment, $2\text{–}4\times10^6$ DP T cells were sorted from Cre-ERT2; $Bcl11b^{flox/flox}$ thymocytes and injected intravenously into each $Rag2^{-/-}Il2rg^{-/-}$ recipient mouse without irradiation. Two weeks later, 5×10^4 B16F10 melanoma cells were injected intravenously and the lung colonies were enumerated 14 days after tumour inoculation.

Calcium Flux Experiments

[0296] flox/flox or flox/+ mice were treated with Tamoxifen to derive in vivo-reprogrammed ITNK as described above and splenocytes were analyzed 4-5 weeks later. Splenocytes were either stained directly with antibodies to NKp46, NK1.1, CD8 and CD3 for phenotyping, or loaded with 2 μM Indo-1 (Invitrogen), washed and stained with antibodies to NKp46, NK1.1 and CD8. Data was then acquired using an LSR II flow cytometer gating on lymphocytes, measuring Indo-1 (violet)/Indo-1 (blue) ratio against time. Unstimulated cells were run to establish the baseline Indo-1 (violet)/Indo-1 (blue) fluorescence before acquisition was interrupted, anti-CD3 (145-2C11; μg/ml) added and acquisition continued. Acquisition was interrupted again and cross-linking anti-hamster IgG secondary antibody was added before continuing. Ionomycin (1 μg/ml) was added at the end of the acquisiton to serve as a positive control.

[0297] 1.1.12 Gene Expression Analysis

[0298] RNA was extracted using the RNAqueous Micro Kit (Ambion) from FACS sorted cells. Quality and quantity of RNA samples was tested with Bioanalyzer. Total RNA was amplified using the Illumina Total Prep RNA Amplification Kit (Ambion) according to the manufacture's instructions. The biotinlated cRNA (1500 ng per sample) was applied to Illumina Mouse-6 Expression BeadChips and hybridized overnight at 58° C. Chips were washed, detected and scanned according to the manufacture's instruction and the scanner output imported into BeadStudio software (Illumina).

Chromatin Immunoprecipitation

[0299] Chromatin immunoprecipitation was performed as previously described (38). Control IgG and the CSL antibody were purchased from Abcam. Genomic DNA was purified with Qiaquick PCR purification kit (QIAGEN) and specific genomic DNA regions were quantified by real-time quantitative PCR with Taqman (ABI) or SYBR Green (Invitrogen). Input DNA was used as a standard curve to quantify concentration of DNA recovered after IP. The amount of DNA recovered from each ChIP sample was presented as relative to the control IgG. Primers used in this assay are listed in table 4.

TABLE 1

ITNK vs. DN3

Column ID	p-value (ITNK vs. DN3)	Ratio (ITNK vs. DN3)	Fold-Change (ITNK vs. DN3)
FCER1G	1.61E−08	38.85426542	38.8542
ROG	3.56E−09	38.51902069	38.519
UPP1	1.59E−11	27.95435613	27.9543
IFITM1	3.53E−06	27.42649015	27.4265
XCL1	1.21E−06	25.36912071	25.3691
SERPINA3G	7.75E−08	21.14875825	21.1487
SCIN	1.90E−08	20.78544021	20.7854
NKG7	1.76E−08	20.18204202	20.182
AQP9	1.71E−07	18.25220532	18.2522
KLRD1	7.30E−09	17.17811645	17.1781
LGALS3	7.17E−09	15.91702772	15.917
AVIL	9.13E−07	13.61854192	13.6185
IFITM3	1.54E−07	13.57143051	13.5714
TYROBP	1.57E−09	13.52446102	13.5245
GADD45G	4.52E−08	13.52446102	13.5245
CD160	3.07E−07	12.64065893	12.6407
IFITM2	4.83E−06	11.27456728	11.2746
CTSW	3.28E−06	9.798061943	9.79809
9130404D14RIK	6.12E−09	9.679979866	9.67995
LOC270152	2.41E−07	9.530162966	9.53016
BC025206	1.38E−08	9.063060777	9.06307
VIM	3.49E−08	8.891971439	8.89195
NFIL3	2.83E−06	8.426871608	8.42689
AMICA1	9.38E−08	8.267810932	8.26778
LTA	1.13E−11	8.210652501	8.21067
GLRX1	2.07E−06	8.027743883	8.02777
LITAF	1.96E−07	7.727497527	7.72749
CCR5	6.07E−09	7.323324789	7.32333
LMNA	5.83E−08	7.235052382	7.23503
BC049975	2.32E−08	6.988559728	6.98858
P2RY14	1.44E−07	6.797495803	6.79748

TABLE 1-continued

WBSCR5	8.76E−08	6.486766995	6.48677
LAG3	8.41E−08	6.190263953	6.19026
LY6A	1.40E−05	5.989781433	5.98977
E030006K04RIK	6.54E−09	5.948379959	5.94839
9130211I03RIK	4.39E−06	5.87668367	5.87667
1300002F13RIK	7.18E−06	5.866513355	5.8665
LOC381140	2.90E−07	5.84620961	5.8462
GPR114	2.07E−05	5.78573123	5.78573
2310067E08RIK	3.25E−07	5.725901114	5.72589
CDKN2B	6.18E−06	5.686341408	5.68634
IDB2	1.14E−08	5.637296353	5.63728
GOLPH2	4.35E−09	5.608053164	5.60805
PLCG2	2.95E−09	5.550036353	5.55005
1500031H04RIK	1.83E−07	5.492634377	5.49264
1110018K11RIK	7.30E−10	5.388947269	5.38893
CD9	9.16E−09	5.388947269	5.38893
LOC381319	6.47E−06	5.323963158	5.32396
SYTL2	3.83E−10	5.250666835	5.25066
SLC2A6	1.27E−07	5.223432317	5.22344
OSBPL3	3.09E−11	5.187367722	5.18736
2210411K11RIK	2.39E−06	5.080603779	5.0806
LRRK1	1.15E−07	5.045510505	5.04551
S100A6	3.80E−07	4.967438441	4.96743
KLRE1	8.01E−08	4.933107068	4.93312
PGLYRP1	6.39E−06	4.933107068	4.93312
GLRX	6.59E−06	4.873650608	4.87364
MYO1F	8.46E−09	4.839966507	4.83998
LOC269941	9.61E−10	4.831594764	4.8316
TRAF1	1.82E−06	4.69946896	4.69948
EMILIN2	0.000282473	4.691333699	4.69134
TNFRSF9	1.19E−07	4.67510367	4.67511
CD52	9.20E−05	4.618745641	4.61874
PLSCR1	2.75E−09	4.602758894	4.60276
BHLHB2	5.80E−06	4.570968863	4.57097
S100A1	8.07E−08	4.55514458	4.55515
LGALS1	4.96E−07	4.523699663	4.52369
2310046K01RIK	3.01E−06	4.4691539	4.46915
CAPG	5.03E−09	4.453688322	4.45369
C330008K14RIK	4.31E−07	4.430601277	4.43059
TNFRSF11B	0.000351472	4.384772562	4.38477
CCL4	8.44E−05	4.362031136	4.36203
SIAT10	6.50E−06	4.339411402	4.33941
HBA-A1	1.28E−06	4.301981923	4.30198
ROM1	1.73E−08	4.272262761	4.27226
1190002C06RIK	2.57E−07	4.198875541	4.19887
F2R	2.39E−05	4.184345527	4.18434
RGS1	5.76E−05	4.184345527	4.18434
CD69	2.26E−05	4.177091992	4.17709
CISH	1.63E−06	4.155429692	4.15544
DAPK2	9.71E−09	4.133888377	4.13389
SH3BP2	3.58E−08	4.098226288	4.09823
GCNT1	2.49E−08	4.069921247	4.06992
HAVCR2	5.18E−05	4.062877086	4.06287
DUSP6	3.27E−09	4.041808467	4.04181
CTNNA1	4.08E−08	3.993068034	3.99307
BC024955	6.91E−10	3.917681673	3.91768
ITGB7	0.000152314	3.917681673	3.91768
MLKL	7.92E−08	3.877156183	3.87716
SERPINE2	2.49E−05	3.870448353	3.87045
LY6G5B	7.00E−06	3.863748764	3.86375
PPP3CC	5.92E−09	3.850374449	3.85038
LOC218482	1.61E−08	3.837062959	3.83706
A430006M23RIK	3.38E−06	3.7776762	3.77768
2410008K03RIK	3.43E−08	3.732137059	3.73213
FURIN	1.27E−06	3.732137059	3.73213
F2RL2	9.14E−05	3.732137059	3.73213
GPR18	2.97E−06	3.712779387	3.71278
HGFAC	7.05E−05	3.70635306	3.70635
S100A10	4.21E−06	3.693525988	3.69353
APOB48R	4.85E−06	3.68074675	3.68075
OSM	1.62E−05	3.68074675	3.68075
AIM1L	2.96E−06	3.674376734	3.67438
IL18R1	3.56E−07	3.661662395	3.66167
NT5E	2.59E−08	3.655331484	3.65533
IFNG	3.30E−06	3.63637686	3.63637
H2-Q8	0.000127421	3.630080297	3.63008

TABLE 1-continued

FXYD4	1.04E−07	3.617513104	3.61752
PILRB	3.51E−05	3.592534713	3.59253
PLP2	3.87E−08	3.580097522	3.5801
MT1	0.000577989	3.580097522	3.5801
DOK2	7.55E−08	3.567707962	3.56771
0610037M15RIK	7.75E−06	3.549220591	3.54922
2310047C17RIK	4.43E−05	3.549220591	3.54922
S100A11	2.00E−07	3.530811628	3.53081
FES	1.98E−08	3.518599316	3.5186
BC029169	3.49E−07	3.500346534	3.50035
TNF	5.66E−10	3.482197266	3.4822
LRP12	7.21E−05	3.482197266	3.4822
IER3	0.000215344	3.476169123	3.47617
NAPSA	2.95E−05	3.470149772	3.47015
ANXA2	3.41E−06	3.434266424	3.43426
PRSS19	1.77E−05	3.428320671	3.42832
OSTF1	5.84E−09	3.381119827	3.38112
GVIN1	0.000422456	3.36941982	3.36942
1110007C02RIK	2.44E−08	3.340348064	3.34035
MAPKAPK3	1.77E−07	3.311532412	3.31153
CD244	7.87E−08	3.277280906	3.27728
F630022B06RIK	4.89E−06	3.271608977	3.27161
ID2	1.03E−07	3.265945981	3.26594
GPR68	6.05E−06	3.265945981	3.26594
GLIPR1	4.48E−07	3.260291927	3.26029
PDGFA	1.76E−06	3.254646822	3.25464
PKP3	1.71E−08	3.254646822	3.25464
D10BWG1379E	5.49E−08	3.254646822	3.25464
SLC39A4	4.73E−08	3.215403067	3.2154
TES	1.92E−07	3.182149415	3.18215
EGR1	0.0012963	3.182149415	3.18215
1110004P15RIK	5.91E−05	3.176640258	3.17664
B4GALNT4	4.86E−07	3.149229384	3.14923
CDKN1A	0.000553492	3.149229384	3.14923
D2ERTD217E	7.91E−08	3.079083172	3.07908
NCF4	5.37E−06	3.073754745	3.07375
LOC381924	1.33E−06	3.047229002	3.04723
170002G04RIK	2.02E−06	3.041954638	3.04196
AA467197	0.00100238	3.03668928	3.03669
SGK	5.37E−08	3.020947248	3.02095
BC021614	5.58E−05	3.015717922	3.01571
LOC385953	1.27E−10	3.005277267	3.00528
CDKN2A	8.87E−06	2.989697502	2.9897
2610009E16RIK	2.28E−05	2.953651304	2.95365
HIST1H1C	0.00103646	2.928171942	2.92817
DCI	1.02E−08	2.912989018	2.91299
NFKB1	1.21E−07	2.902909296	2.90291
TPST2	2.16E−06	2.902909296	2.90291
TRF	2.76E−06	2.89788715	2.89788
HK2	3.64E−06	2.887861452	2.88786
PDZK1	5.44E−10	2.88285795	2.88286
GNG2	6.86E−09	2.872886274	2.87288
S100A4	8.62E−08	2.848102167	2.8481
ZFP608	8.21E−08	2.838248233	2.83825
2210008N01RIK	6.64E−08	2.83333475	2.83333
SH3BGRL3	4.55E−05	2.828430249	2.82843
MYO1G	2.01E−06	2.823526754	2.82353
1110019C08RIK	1.34E−05	2.818640232	2.81864
S100A13	8.29E−05	2.818640232	2.81864
PPAP2C	3.95E−06	2.813762676	2.81376
MYO1E	7.25E−08	2.808886192	2.80889
IFI30	1.07E−06	2.799168087	2.79917
LTB4R1	0.000868775	2.784654327	2.78466
TMEM126A	3.48E−08	2.775025877	2.77502
1110020C13RIK	7.24E−08	2.775025877	2.77502
CD7	7.40E−06	2.775025877	2.77502
4933439K08RIK	1.06E−08	2.751084615	2.75108
E030003N15RIK	2.16E−05	2.732083678	2.73208
CCL5	0.0117888	2.732083678	2.73208
7420404O03RIK	1.10E−05	2.722629407	2.72263
4930486L24RIK	1.95E−06	2.717915685	2.71791
BC022224	9.36E−07	2.713210895	2.71321
5330403J18RIK	5.98E−06	2.708507694	2.70851
FXYD5	4.01E−06	2.703820769	2.70382
A430038C16RIK	2.01E−06	2.699142752	2.69914
GPC1	0.00472373	2.675857345	2.67586

TABLE 1-continued

AHNAK	7.45E−07	2.666595557	2.6666
EMP1	0.000268268	2.666595557	2.6666
CX3CR1	3.25E−09	2.661981579	2.66198
2810032E02RIK	4.29E−07	2.661981579	2.66198
LOC327957	0.000272484	2.657369417	2.65737
FCGR3	3.00E−05	2.625333417	2.62533
CLNK	1.01E−08	2.620785449	2.62079
HVCN1	0.000222656	2.620785449	2.62079
BSCL2	5.79E−06	2.616246367	2.61625
LGALS3BP	6.49E−06	2.616246367	2.61625
CYP51	7.38E−07	2.607194815	2.6072
PIM3	6.98E−05	2.593677134	2.59368
BC03881	7.63E−09	2.584707321	2.58471
LOC383981	0.00132323	2.584707321	2.58471
PDLIM7	5.33E−07	2.580232324	2.58023
SLC24A3	6.09E−05	2.580232324	2.58023
HHEX	5.95E−07	2.575766162	2.57576
D930046M13RIK	1.74E−05	2.575766162	2.57576
BC087945	7.68E−07	2.57130221	2.5713
9830144J08RIK	1.24E−07	2.566853705	2.56685
DP1	1.29E−06	2.566853705	2.56685
E130012A19RIK	1.31E−05	2.562407433	2.56241
4631423F02RIK	7.66E−05	2.540301889	2.5403
4930555L03RIK	4.40E−08	2.535902033	2.5359
FOSL2	6.92E−06	2.535902033	2.5359
ZFP296	2.68E−09	2.518384205	2.51839
F730045P10RIK	0.000293728	2.518384205	2.51839
PEA15	5.59E−08	2.509674796	2.50967
ITGAE	0.000534865	2.50533009	2.50533
A530050E01RIK	1.01E−05	2.50533009	2.50533
SCL0001419.1_32	1.67E−07	2.500994145	2.50099
SPP1	0.00129868	2.496660716	2.49666
ASB2	2.54E−05	2.492336067	2.49234
CCNG1	4.25E−05	2.492336067	2.49234
TUBA6	1.47E−06	2.483713052	2.48372
SDF2L1	7.56E−05	2.483713052	2.48372
RHOF	9.16E−06	2.47941466	2.47942
1110030J09RIK	2.72E−10	2.45802311	2.45803
EGR3	0.00128127	2.45802311	2.45803
CXCR3	5.82E−05	2.453770955	2.45377
ALDOA	0.000483723	2.449521486	2.44952
GCNT2	3.22E−08	2.44528073	2.44528
MVP	4.37E−07	2.44528073	2.44528
C130027E04RIK	5.53E−07	2.44528073	2.44528
SEC61B	1.47E−09	2.436819366	2.43682
E430036I04RIK	2.87E−07	2.43259877	2.4326
AI481100	9.30E−05	2.424183656	2.42419
CD63	0.000436295	2.41998911	2.41999
DEGS	4.85E−07	2.415797382	2.4158
LOC385699	1.78E−05	2.415797382	2.4158
EG331493	3.45E−06	2.415797382	2.4158
A930008A22RIK	6.04E−07	2.411614335	2.41162
4930504E06RIK	0.000436715	2.411614335	2.41162
LOC212399	1.73E−06	2.407439952	2.40744
AI850995	3.62E−06	2.407439952	2.40744
CTGF	0.000743838	2.407439952	2.40744
KIRL2	3.13E−06	2.394957178	2.39496
1700017I11RIK	6.32E−08	2.390811633	2.39081
2310037P21RIK	2.51E−06	2.386669022	2.38667
UAP1L1	3.29E−06	2.382540741	2.38254
D14ERTD449E	0.000867516	2.382540741	2.38254
BC023892	4.42E−09	2.378415405	2.37841
AA175286	0.000409847	2.361983405	2.36199
PPIB	5.05E−09	2.357895531	2.3579
GPR34	1.68E−07	2.357895531	2.3579
IRAK2	7.34E−05	2.357895531	2.3579
SH2D1B1	0.000605208	2.353810702	2.35381
HSD11B1	6.66E−05	2.353810702	2.35381
LOC328703	3.58E−06	2.349740001	2.34974
BC004728	5.49E−07	2.349740001	2.34974
LOC215405	4.47E−05	2.349740001	2.34974
RAB3D	1.16E−05	2.337551835	2.33755
SULT2B1	0.000164735	2.337551835	2.33755
EVI2A	2.55E−07	2.333509902	2.33351
TSPO	2.22E−06	2.333509902	2.33351
TNFRSF18	2.31E−05	2.333509902	2.33351

TABLE 1-continued

EG630499	0.000147665	2.329465644	2.32947
SERPINB6A	0.00133703	2.32543538	2.32543
SYPL	6.57E−07	2.321408259	2.32141
8030402P03RIK	0.000483123	2.321408259	2.32141
HAAO	3.04E−05	2.317389692	2.31739
NRGN	5.54E−05	2.313374311	2.31338
TAF9B	1.33E−06	2.309372821	2.30937
9930117H01RIK	6.56E−05	2.309372821	2.30937
GBP2	0.000466109	2.309372821	2.30937
AW212394	7.13E−06	2.30537452	2.30537
KIT	0.000138895	2.301379447	2.30138
A630086H07RIK	3.07E−05	2.297398197	2.2974
ANK	1.14E−07	2.293420178	2.29342
BATF	6.24E−06	2.293420178	2.29342
TIAM1	1.13E−07	2.289450669	2.28945
TCRD-V1	6.14E−06	2.285484431	2.28548
ARL6IP5	2.09E−07	2.285484431	2.28548
EHD4	3.43E−05	2.285484431	2.28548
N4WBP5-PENDING	9.39E−08	2.281526706	2.28153
B3GNT8	1.44E−07	2.281526706	2.28153
BLR1	8.56E−06	2.281526706	2.28153
NDFIP1	2.11E−06	2.261844715	2.26184
PRDX4	1.09E−05	2.261844715	2.26184
SNAG1	4.20E−07	2.250118694	2.25012
B4GALNT2	0.000956597	2.250118694	2.25012
TRPM6	9.96E−08	2.238448487	2.23845
CXCL9	0.00820156	2.230703854	2.23071
0610009O03RIK	8.23E−09	2.222987918	2.22299
PRR7	1.26E−06	2.207632226	2.20763
A630077B13RIK	3.14E−05	2.207632226	2.20763
SLC19A2	4.63E−05	2.207632226	2.20763
2810440J20RIK	7.67E−07	2.192381474	2.19238
MED10	3.04E−06	2.192381474	2.19238
COMT	8.15E−09	2.188586086	2.18859
PLTP	6.19E−07	2.188586086	2.18859
2310010I15RIK	1.21E−08	2.181015568	2.18102
0610039P13RIK	0.000646447	2.181015568	2.18102
VPS29	1.09E−07	2.177240435	2.17724
AI847670	1.60E−06	2.16595082	2.16595
ASAH1	3.80E−08	2.162199562	2.1622
B830021E24RIK	1.42E−05	2.162199562	2.1622
SRGAP2	4.27E−06	2.158456617	2.15846
IQGAP2	0.000141764	2.158456617	2.15846
LASP1	2.73E−06	2.154717323	2.15472
CORO1C	7.53E−07	2.150990962	2.15099
H2-Q6	3.02E−06	2.150990962	2.15099
9130604K18RIK	4.57E−06	2.147263635	2.14726
FNBP1	2.88E−08	2.143549203	2.14355
TMPIT	3.47E−06	2.139833863	2.13984
H2-Q7	5.84E−05	2.136131381	2.13613
0610007H07RIK	2.48E−06	2.132432594	2.13243
CCND2	3.28E−06	2.125055251	2.12505
SERTAD1	0.000141861	2.121376689	2.12138
RAB19	7.99E−06	2.114035292	2.11404
BAG3	0.00480518	2.110376934	2.11038
VTI1B	1.30E−05	2.10672234	2.10672
CAPN2	2.23E−06	2.103075959	2.10307
2310057H16RIK	6.11E−05	2.099433363	2.09943
STX11	0.000109261	2.095798971	2.0958
FTL1	0.00023523	2.095798971	2.0958
ARF6	3.80E−07	2.092168386	2.09217
2900026A02RIK	2.85E−07	2.088545996	2.08855
CSTB	3.81E−05	2.088545996	2.08855
LOC383189	5.48E−07	2.084931781	2.08493
CCL3	0.00511685	2.084931781	2.08493
GLIPR2	6.01E−05	2.084931781	2.08493
C330023F11RIK	1.81E−06	2.081321389	2.08132
SIRT3	2.69E−06	2.081321389	2.08132
CAPNS1	3.44E−08	2.077719163	2.07772
RBMS1	1.72E−07	2.077719163	2.07772
1110008P14RIK	2.41E−07	2.077719163	2.07772
MINA	1.96E−08	2.07412078	2.07412
CCDC132	2.24E−06	2.07412078	2.07412
LOC234582	1.09E−06	2.066944188	2.06695
KCTD10	9.73E−05	2.066944188	2.06695
LOC240672	5.49E−06	2.056229656	2.05623

TABLE 1-continued

A230057G18RIK	9.28E−07	2.056229656	2.05623
ELOVL1	3.81E−06	2.056229656	2.05623
STX7	5.97E−06	2.052667339	2.05267
BC017612	5.16E−06	2.052667339	2.05267
ZBTB32	3.61E−05	2.052667339	2.05267
H47	3.72E−06	2.049113144	2.04911
TNFRSF22	7.52E−05	2.049113144	2.04911
AI115600	1.65E−06	2.045567052	2.04557
MYL6	1.28E−07	2.045567052	2.04557
H2-GS17	0.000428176	2.042024872	2.04202
CAPZB	5.20E−07	2.038490783	2.03849
SC4MOL	3.38E−06	2.038490783	2.03849
FHL2	6.33E−07	2.034960624	2.03496
3010031K01RIK	4.37E−08	2.034960624	2.03496
A330042I21RIK	1.18E−06	2.034960624	2.03496
D15MGI27	2.70E−05	2.034960624	2.03496
RAB4A	4.71E−08	2.024406242	2.02441
DCXR	0.000151877	2.024406242	2.02441
AIM1	0.000114024	2.024406242	2.02441
SEMA4A	7.97E−05	2.017402111	2.0174
XBP1	0.000154152	2.017402111	2.0174
LOC383099	0.000417017	2.013912105	2.01391
JUNB	0.00325781	2.013912105	2.01391
HRMT1L1	7.88E−06	2.01042607	2.01042
GPR97	0.000165473	2.01042607	2.01042
COTL1	0.000539041	2.01042607	2.01042
2310061N23RIK	0.025362	2.006944026	2.00694
9130227C08RIK	4.47E−06	2.00347001	2.00347
AI840980	0.000521047	2.00347001	2.00347
DYRK3	0.000241993	2.00347001	2.00347
CASP1	0.00573828	2.00347001	2.00347
TRBV11_AE000663_T_CELL_RECEPTOR_BETA_VARIABLE_11_	0.000105474	0.5	−2
C920004C08RIK	0.0113298	0.5	−2
A930023F05RIK	1.93E−06	0.499134003	−2.00347
PLEKHG2	1.52E−05	0.499134003	−2.00347
ABHD8	7.47E−05	0.499134003	−2.00347
3110013H01RIK	0.00068445	0.499134003	−2.00347
E030007N04RIK	3.23E−07	0.496546519	−2.01391
PRKCD	1.24E−06	0.495687519	−2.0174
9130430L19RIK	1.04E−05	0.494829037	−2.0209
6030443O07RIK	1.78E−05	0.494829037	−2.0209
A130062D16RIK	1.86E−06	0.493971083	−2.02441
5930416I19RIK	2.26E−06	0.493971083	−2.02441
FYB	9.40E−06	0.493971083	−2.02441
AA408556	0.000447224	0.491410151	−2.03496
TRBV31_X03277_T_CELL_RECEPTOR_BETA_VARIABLE_31_33	0.00870127	0.491410151	−2.03496
A130038J17RIK	8.99E−05	0.490559188	−2.03849
AJ237586	1.68E−05	0.490559188	−2.03849
ZFP260	3.69E−06	0.490559188	−2.03849
0710008K08RIK	9.80E−06	0.489711168	−2.04202
ANP32E	0.000183335	0.485486385	−2.05979
4921518A06RIK	8.35E−06	0.484644053	−2.06337
4933421G18RIK	1.04E−05	0.484644053	−2.06337
3110018A08RIK	0.00338526	0.484644053	−2.06337
C730009F21RIK	1.18E−07	0.48380464	−2.06695
OLFML3	1.61E−05	0.482132181	−2.07412
A330103N21RIK	9.39E−05	0.481296806	−2.07772
H2-T9	0.000542267	0.481296806	−2.07772
LOC386360	0.00269607	0.481296806	−2.07772
ILVBL	4.13E−05	0.478801082	−2.08855
SBK	5.91E−07	0.477972631	−2.09217
6330403M23RIK	6.88E−08	0.476319763	−2.09943
C230075L19RIK	4.42E−07	0.475495347	−2.10307
MSH6	0.00037102	0.475495347	−2.10307
CXCL12	0.00745872	0.475495347	−2.10307
BC035291	1.11E−05	0.474671527	−2.10672
MMP2	0.000824671	0.473848312	−2.11038
GM525	0.000141229	0.473027946	−2.11404
STK4	2.73E−05	0.472210417	−2.1177
A130093I21RIK	7.45E−05	0.471391264	−2.12138
A230013K13RIK	3.45E−06	0.471391264	−2.12138
0610041G09RIK	0.00831324	0.471391264	−2.12138
TRIM28	3.45E−05	0.470577163	−2.12505
B230342M21RIK	7.67E−06	0.46894857	−2.13243
1190002H23RIK	5.23E−05	0.464902208	−2.15099
TSPAN32	9.87E−06	0.462491906	−2.1622

TABLE 1-continued

2610019F03RIK	3.67E−05	0.462491906	−2.1622
H2-OB	1.61E−05	0.461691175	−2.16595
4732481H14RIK	1.72E−05	0.460891087	−2.16971
COL5A1	0.00288136	0.460891087	−2.16971
LDH2	8.49E−05	0.459297092	−2.17724
6720418B01RIK	7.62E−06	0.458501068	−2.18102
6430510M02RIK	1.62E−06	0.458501068	−2.18102
TNFRSF7	0.000102955	0.458501068	−2.18102
CRYL1	8.89E−07	0.458501068	−2.18102
B230345P09RIK	5.94E−05	0.458501068	−2.18102
CTLA4	0.000629703	0.456915183	−2.18859
RGL2	2.61E−06	0.45533401	−2.19619
1810015C11RIK	3.91E−09	0.452974457	−2.20763
F730003H07RIK	0.00244488	0.452974457	−2.20763
CD97	0.000238915	0.452189956	−2.21146
LLGL1	1.90E−06	0.450625017	−2.21914
LOX	0.000702165	0.450625017	−2.21914
PDXP	3.27E−05	0.449844579	−2.22299
TRIB2	4.12E−06	0.449066839	−2.22684
2210008I11RIK	0.000393721	0.449066839	−2.22684
H2-AB1	0.00426068	0.449066839	−2.22684
SLC29A1	8.67E−06	0.448287765	−2.23071
ITPR2	5.30E−06	0.446737698	−2.23845
TPST1	9.65E−06	0.445964689	−2.24233
RPS6KL1	9.39E−07	0.442883526	−2.25793
RIL-PENDING	1.64E−06	0.441351064	−2.26577
TTC3	8.83E−09	0.439825301	−2.27363
MAPK1	2.50E−07	0.439062514	−2.27758
H2-EB1	0.00320736	0.438302367	−2.28153
CD3D	0.000186864	0.435274658	−2.2974
TRBV8_AE000663_T_CELL_RECEPTOR_BETA_VARIABLE_8_27	0.000766092	0.435274658	−2.2974
PPP1R1C	6.89E−07	0.434521896	−2.30138
PITPNM2	5.21E−07	0.432267937	−2.31338
2210419D22RIK	1.12E−07	0.431519943	−2.31739
RAPGEF3	1.11E−07	0.430772677	−2.32141
SATB1	5.66E−06	0.427056598	−2.34161
C530015C18	9.54E−09	0.426317427	−2.34567
5830496L11RIK	1.02E−06	0.424843127	−2.35381
BCL7A	3.37E−07	0.424843127	−2.35381
GLDC	0.000448781	0.424106196	−2.3579
CD27	0.000101099	0.423371818	−2.36199
A830080H07RIK	1.99E−05	0.423371818	−2.36199
ART4	9.75E−06	0.422639978	−2.36608
SCL000121.1_106	6.92E−06	0.419720131	−2.38254
4932414K18RIK	2.36E−06	0.418993828	−2.38667
ACVR2B	4.27E−08	0.417543508	−2.39496
AI481316	4.04E−07	0.417543508	−2.39496
POU6F1	8.86E−07	0.417543508	−2.39496
NCK2	0.000270785	0.412508921	−2.42419
1110046J11RIK	5.70E−05	0.411082792	−2.4326
ETS2	1.91E−06	0.408243248	−2.44952
FBP1	0.00205479	0.408243248	−2.44952
TPCN1	3.56E−08	0.407536159	−2.45377
TBXA2R	3.11E−06	0.405422937	−2.46656
5430417L22RIK	5.18E−07	0.404020815	−2.47512
PPARGC1B	1.79E−07	0.404020815	−2.47512
TCF7	1.40E−05	0.398458762	−2.50967
DNTT	0.00027177	0.397767728	−2.51403
LOC386545	0.00608272	0.394337316	−2.5359
SOX4	1.85E−07	0.390257609	−2.56241
GPR83	1.40E−05	0.388908334	−2.5713
HIBADH	2.18E−08	0.387562349	−2.58023
IGH-6	2.87E−05	0.386221173	−2.58919
LOC381739	1.61E−06	0.382889437	−2.61172
DAP3	2.30E−08	0.380904496	−2.62533
DGKA	8.26E−05	0.378928542	−2.63902
SNAI3	4.51E−07	0.376964456	−2.65277
SLC5A9	2.33E−05	0.376964456	−2.65277
2410008J05RIK	1.24E−06	0.376311917	−2.65737
NAV1	2.19E−06	0.376311917	−2.65737
HDAC7A	2.22E−06	0.376311917	−2.65737
A130092J06RIK	6.62E−06	0.375660223	−2.66198
SLA	2.73E−05	0.373064727	−2.6805
MTF2	2.39E−06	0.371130501	−2.69447
C230098O21RIK	6.12E−05	0.370488378	−2.69914
GFI1	2.05E−06	0.368567122	−2.71321

TABLE 1-continued

EPHX1	2.39E−08	0.36792977	−2.71791
BRD3	2.92E−06	0.36792977	−2.71791
AQP11	4.62E−07	0.364754502	−2.74157
IL17RB	2.04E−07	0.362236156	−2.76063
RAMP1	0.000135079	0.361608725	−2.76542
NISCH	3.31E−07	0.361608725	−2.76542
BGN	0.0025721	0.36098216	−2.77022
TXNIP	0.000742272	0.359732934	−2.77984
COL6A1	0.00166573	0.359110268	−2.78466
CCL9	0.000264226	0.356012517	−2.80889
DPP4	1.63E−06	0.354166593	−2.82353
MLL	1.54E−07	0.352329781	−2.83825
C3	0.0001376	0.350503323	−2.85304
MARCKS	4.55E−06	0.349896256	−2.85799
TRBV1__AE000663__T__CELL__RECEPTOR__BETA__VARIABLE__1__20	0.00160689	0.34808276	−2.87288
3830612M24	2.00E−06	0.344481916	−2.90291
PP11R	0.000186213	0.344481916	−2.90291
2510015F01RIK	0.000255295	0.342695782	−2.91804
PARD6G	1.26E−07	0.327598181	−3.05252
NOTCH3	3.24E−06	0.327598181	−3.05252
H2-T10	0.000761011	0.327598181	−3.05252
LMAN2L	1.87E−07	0.327031438	−3.05781
DTX1	2.93E−07	0.324772335	−3.07908
TMEM108	2.25E−05	0.324210062	−3.08442
ETS1	5.90E−07	0.322528624	−3.1005
SH2D1A	2.22E−06	0.320856301	−3.11666
9626100__15	0.00161165	0.314252942	−3.18215
CD8B	3.35E−05	0.313166457	−3.19319
ACAS2L	7.23E−06	0.30992664	−3.22657
LOC434197	1.33E−06	0.305659904	−3.27161
9626100__224	0.00113435	0.305131084	−3.27728
FRAT2	2.01E−06	0.302498639	−3.3058
NRP	8.11E−07	0.299888741	−3.33457
G22P1	8.46E−08	0.296273472	−3.37526
RNPEPL1	4.01E−09	0.295759985	−3.38112
9626958__317	0.00188407	0.293717673	−3.40463
H19	0.000268847	0.283221131	−3.53081
ACTN1	2.58E−07	0.278838137	−3.58631
SLC16A5	3.62E−09	0.275476023	−3.63008
CD2	1.50E−06	0.274999519	−3.63637
PRKCB	2.18E−07	0.272154758	−3.67438
ST6GAL1	7.38E−08	0.268873581	−3.71922
PRELP	1.65E−05	0.268408098	−3.72567
CDCA7	4.57E−05	0.267943507	−3.73213
PDLIM4	4.93E−06	0.26701628	−3.74509
CD6	1.61E−09	0.264712734	−3.77768
ALDH2	7.40E−07	0.248703632	−4.02085
CD81	6.28E−06	0.247413906	−4.04181
9430068D06RIK	3.23E−10	0.239816205	−4.16986
H2-BL	9.51E−06	0.232854352	−4.29453
AI132321	1.19E−06	0.20877219	−4.78991
LY6D	2.50E−08	0.184923553	−5.40764
COX6A2	0.000397718	0.158219587	−6.32033
BCL11B	1.64E−08	0.150204503	−6.65759
LOC382896	6.81E−09	0.112266584	−8.90737

LAK vs. DN3

Column ID	p-value (LAK vs. DN3)	Ratio (LAK vs. DN3)	Fold-Change (LAK vs. DN3)
GZMD	2.97E−07	79.3411511	79.3413
FCER1G	8.83E−09	50.3005458	50.3005
ROG	3.50E−09	38.7869009	38.787
CCL4	4.38E−08	38.5859038	38.5858
KLRE1	6.58E−11	35.1998648	35.1999
MT1	1.56E−07	35.0174387	35.0174
SPP1	2.96E−08	32.5029903	32.503
AVIL	8.90E−08	30.6432634	30.6433
TYROBP	1.95E−10	26.8620793	26.8621
GZME	6.58E−10	26.1728718	26.1729
XCL1	1.12E−06	26.1275337	26.1276
ASB2	4.61E−10	25.8125134	25.8125
KLRA7	9.89E−11	22.7059601	22.706
PRF1	9.89E−08	22.2386788	22.2387
KLRD1	4.72E−09	19.8353271	19.8353

TABLE 1-continued

LGALS3	4.28E−09	18.7979112	18.7979
GZMG	6.92E−08	18.0634207	18.0634
KLRA18	6.87E−08	16.6794542	16.6795
SERPINA3G	1.62E−07	16.5069346	16.5069
NKG7	3.26E−08	16.4783466	16.4784
LTB4R1	3.28E−07	16.1392364	16.1392
GADD45G	2.57E−08	16.0834021	16.0834
CTSG	5.88E−06	15.9446402	15.9446
CCL3	2.43E−07	15.5624826	15.5625
NFIL3	3.46E−07	15.3482283	15.3482
AQP9	3.11E−07	15.0323722	15.0324
1300002F13RIK	2.22E−07	14.4450222	14.445
KLRA4	9.74E−08	14.2461507	14.2461
LITAF	2.42E−08	13.4543507	13.4543
KLRA3	5.93E−09	13.2691108	13.2691
LRRK1	1.90E−09	13.2232104	13.2232
KLRG1	1.44E−07	13.2003094	13.2003
EMILIN2	5.64E−06	12.8393439	12.8393
1110007C02RIK	3.19E−11	12.7727787	12.7728
TNFRSF11B	5.09E−06	12.7727787	12.7728
LOC381140	1.29E−08	12.4666362	12.4666
HAVCR2	4.62E−07	12.0211235	12.0211
LOC327957	1.39E−07	12.0003072	12.0003
PDGFA	2.78E−09	11.9174171	11.9174
SCIN	1.18E−07	11.7329992	11.733
BC049975	2.96E−09	11.6519561	11.652
PGLYRP1	1.73E−07	11.5114671	11.5115
IFITM1	4.19E−05	11.4716392	11.4716
SPEER3	4.84E−05	10.7405848	10.7406
1810044J04RIK	3.59E−09	10.6848908	10.6849
CCR5	1.38E−09	10.519539	10.5195
WBSCR5	1.21E−08	10.4287901	10.4288
DAF1	3.14E−07	10.3388239	10.3388
2210411K11RIK	1.18E−07	10.091021	10.091
P2RY14	3.12E−08	9.81508382	9.81508
BCL2A1B	2.31E−08	9.781099	9.78112
F2R	5.12E−07	9.71354748	9.71356
LOC268288	1.40E−08	9.67997987	9.67995
RGS1	1.42E−06	9.54672166	9.54669
2310057H16RIK	5.56E−09	9.30171989	9.30174
BHLHB2	2.26E−07	9.30171989	9.30174
CTSW	4.35E−06	9.07877654	9.07879
5330403J18RIK	6.45E−09	9.06306078	9.06307
SH2D1B1	3.54E−07	8.89197144	8.69195
PLSCR1	1.15E−10	8.86116329	8.86119
1110018K11RIK	7.85E−11	8.69391339	8.69388
ICSBP1	4.16E−07	8.58907298	8.58906
SPEER1-PS1	0.000100291	8.29648312	8.29648
RGS16	6.08E−08	8.22490171	8.22491
EG433016	0.000986707	8.2106525	8.21067
DHRS6	2.02E−06	8.19645258	8.19646
2310067E08RIK	7.56E−08	7.88985759	7.88986
KLRA13	3.45E−10	7.87618635	7.8762
TNFSF6	7.56E−09	7.70077855	7.70076
CCL5	0.000147502	7.38705198	7.38706
E030006K04RIK	2.60E−09	7.23505238	7.23503
CAR2	7.88E−11	6.90431312	6.90432
SERPINE2	1.33E−06	6.90431312	6.90432
IER3	7.22E−06	6.84476752	6.84476
TNFRSF9	1.74E−08	6.83288806	6.83291
SIAT10	6.93E−07	6.7974958	6.79748
GLRX1	4.58E−06	6.64606387	6.64606
DAPK2	7.72E−10	6.61161396	6.6116
F2RL2	5.36E−06	6.55458329	6.55456
IFITM3	2.68E−06	6.46433304	6.46433
MLKL	5.34E−09	6.30938711	6.30939
SYTL2	1.70E−10	6.14749059	6.1475
TMEM119	0.000650503	6.1368518	6.13686
2810025M15RIK	1.47E−08	6.0418945	6.04189
SEPN1	8.40E−07	6.0418945	6.04189
TCRD-V1	7.87E−09	6.01055453	6.01056
OSBPL3	1.46E−11	5.98978143	5.98977
APOB48R	3.32E−07	5.98978143	5.98977
CD52	2.73E−05	5.979395	5.9794
MYO1F	2.88E−09	5.93810123	5.93809
SH3BP2	4.75E−09	5.8970609	5.89706

TABLE 1-continued

RASD2	1.15E−06	5.85634388	5.85634
LOC269941	3.60E−10	5.80581859	5.80582
IFITM2	6.30E−05	5.80581859	5.80582
GPR87	1.21E−06	5.77570622	5.77572
LOC270152	2.10E−06	5.74577255	5.74577
HIST1H1C	2.95E−05	5.71598418	5.71598
AA467197	3.72E−05	5.70606897	5.70608
KLRA1	3.83E−07	5.63729635	5.63728
IDB2	1.24E−08	5.55003635	5.55005
LY6A	2.01E−05	5.54044246	5.54044
FCGR3	2.61E−07	5.51170687	5.51171
GVIN1	3.28E−05	5.51170687	5.51171
A430038C16RIK	2.04E−08	5.42640707	5.42642
ID2	4.68E−09	5.37961235	5.3796
S100A1	3.25E−08	5.37961235	5.3796
CAPG	1.77E−09	5.37028087	5.37029
PPP3CC	8.68E−10	5.33319111	5.33319
1500031H04RIK	2.19E−07	5.30554642	5.30554
PLCG2	3.91E−09	5.25978056	5.25977
NCF4	1.99E−07	5.24158464	5.24157
AI115600	1.22E−09	5.14260442	5.14261
DUSP6	8.20E−10	5.11595308	5.11594
SERPINB6A	9.97E−06	5.09824315	5.09824
BCL2A1D	1.37E−08	4.89905497	4.89904
UPP1	1.21E−08	4.85677374	4.85678
PIM3	1.14E−06	4.85677374	4.85678
AMICA1	1.15E−06	4.84836731	4.84837
SLC2A3	4.16E−06	4.8148915	4.81488
5031436O0RIK	2.13E−06	4.77333804	4.77334
CD160	1.84E−05	4.73215976	4.73216
A430084P05RIK	2.72E−07	4.6913337	4.69134
GOLPH2	1.25E−08	4.61874564	4.61874
CD244	8.49E−09	4.61874564	4.61874
DMWD	1.23E−06	4.57890134	4.5789
AHNAK	1.67E−08	4.57096886	4.57097
TRAF1	2.12E−06	4.57096886	4.57097
TES	1.82E−08	4.55514458	4.55515
CDKN2B	1.88E−05	4.55514458	4.55515
1110004P15RIK	6.74E−06	4.55514458	4.55515
SRGAP2	1.33E−08	4.50803783	4.50804
SULF2	3.60E−09	4.4691539	4.46915
HHEX	1.23E−08	4.38477256	4.38477
LAG3	5.75E−07	4.30198192	4.30198
ALDOA	1.30E−05	4.22074496	4.22075
AI850995	5.34E−08	4.2134358	4.21344
PTER	3.14E−06	4.20615192	4.20615
LOC218482	9.08E−09	4.19887554	4.19887
1190002C06RIK	2.68E−07	4.16986356	4.16986
CTNNA1	3.18E−08	4.15542969	4.15544
CDKN1A	0.000116956	4.14106111	4.14106
2310016C16RIK	9.43E−06	4.12674045	4.12673
A530050E01RIK	2.79E−07	4.08404974	4.08405
LMNA	1.14E−06	4.06287709	4.06287
GLIPR1	1.07E−07	4.03481236	4.03481
9830144J08RIK	4.06E−09	4.02084406	4.02085
PPAP2C	3.54E−07	3.95862446	3.95863
SAT1	1.91E−05	3.95862446	3.95863
SLC2A1	5.83E−05	3.95862446	3.95863
HAK	4.36E−08	3.93809318	3.9381
SCL0003187.1_40	6.01E−07	3.91768167	3.91768
2310046K01RIK	7.02E−06	3.86374876	3.86375
RHOF	3.34E−07	3.84371457	3.84371
LOC212399	4.55E−08	3.82378471	3.82378
CD69	3.83E−05	3.81055447	3.51055
B230343A10RIK	1.49E−08	3.80395916	3.80395
BC022224	7.94E−08	3.7776762	3.77768
KLRB1C	9.87E−09	3.74509393	3.74509
MYO1E	8.79E−09	3.7192142	3.71922
KLRA33	3.61E−06	3.69352599	3.69353
WDFY1	5.95E−08	3.68074675	3.68075
GCNT1	5.12E−08	3.64268203	3.64268
C80638	3.80E−05	3.64268203	3.64268
HGFAC	7.89E−05	3.63637686	3.63637
RASL12	4.28E−07	3.61125556	3.61125
GNG2	1.28E−09	3.58009752	3.5801
SH2D2A	1.04E−06	3.56770796	3.56771

TABLE 1-continued

GPR141	1.94E−05	3.56770796	3.56771
DCI	2.41E−09	3.51859932	3.5186
GLRX	4.20E−05	3.51859932	3.5186
LOC385699	9.48E−07	3.51250628	3.5125
CD72	8.84E−05	3.48824635	3.48824
EGR1	0.000778832	3.48824635	3.48824
2210008N01RIK	1.43E−08	3.45814948	3.45815
ADORA2B	6.64E−07	3.44618438	3.44618
KLRA10	4.19E−08	3.43426642	3.43426
S100A11	2.42E−07	3.43426642	3.43426
F730045P10RIK	3.27E−05	3.43426642	3.43426
PILRB	4.70E−05	3.42832067	3.42832
LGALS1	2.90E−06	3.4046378	3.40463
S100A10	7.33E−06	3.39285533	3.39286
NQO2	4.88E−08	3.38698315	3.38698
CD9	1.56E−07	3.38111983	3.38112
PLP2	5.92E−08	3.36941982	3.36942
SLC39A4	3.40E−08	3.35194782	3.35195
7420404O03RIK	2.40E−06	3.33456712	3.33457
NEDD9	3.25E−07	3.31153241	3.31153
BSPRY	1.11E−06	3.28866234	3.28866
STX11	2.41E−06	3.28866234	3.28866
NFKBIZ	5.41E−07	3.27728091	3.27728
ADAM8	1.09E−06	3.27728091	3.27728
PPP1R3B	1.39E−06	3.27728091	3.27728
SEC61B	1.17E−10	3.26594598	3.26594
S100A6	4.92E−06	3.26594598	3.26594
ANXA2	4.93E−06	3.25464682	3.25464
HK2	1.70E−06	3.19319466	3.19319
PDLIM7	9.37E−08	3.18766755	3.18766
TRIO	4.54E−07	3.17664026	3.17664
ENO1	2.54E−07	3.16016673	3.16017
LOC383981	0.000359161	3.15469355	3.15469
N4WBP5-PENDING	5.19E−09	3.14922938	3.14923
LOC328703	2.98E−07	3.14378411	3.14378
HIC1	5.07E−06	3.11666293	3.11666
AOAH	2.89E−09	3.11126183	3.11126
1110020C13R1K	2.86E−08	3.11126183	3.11126
MAPKAPK3	2.85E−07	3.10587943	3.10588
FTL1	8.72E−06	3.09513198	3.09513
BC046404	6.62E−07	3.07908317	3.07908
E030003N15RIK	8.70E−06	3.07908317	3.07908
VIM	1.02E−05	3.0684259	3.06843
CISH	1.19E−05	3.0684259	3.06843
C230043G09RIK	1.82E−07	3.05252169	3.05252
GPR97	4.15E−06	3.04195464	3.04196
LASP1	1.17E−07	3.03668928	3.03669
9930117H01RIK	6.85E−06	3.03668928	3.03669
CASP1	0.000296727	3.000075	3.00008
D10ERTD438E	3.40E−07	2.97935604	2.97935
TBC1D2B	7.23E−09	2.97419883	2.9742
1700129I15RIK	2.83E−05	2.96390263	2.96391
NDFIP1	1.87E−07	2.95877246	2.95878
5730469M10RIK	9.16E−05	2.95877246	2.95878
JUNB	0.000168611	2.95877246	2.95878
PGK1	2.49E−06	2.9536513	2.95365
LOC240672	1.77E−07	2.94853915	2.94854
BSCL2	2.21E−06	2.94343599	2.94343
IFNG	1.45E−05	2.9383332	2.93834
S100A13	6.20E−05	2.93324807	2.93325
CHN2	1.49E−07	2.92817194	2.92817
CST7	1.31E−07	2.9231048	2.9231
POLD4	1.30E−05	2.91298902	2.91299
BC087945	2.69E−07	2.90794888	2.90794
4631423F02RIK	2.76E−05	2.89286561	2.89287
2310061N23RIK	0.00286637	2.87288627	2.87288
GIPC2	3.20E−05	2.86294236	2.86295
AA175286	9.62E−05	2.84810217	2.8481
TUBA6	4.58E−07	2.83824823	2.83825
KLRB1D	7.14E−11	2.83333475	2.83333
HRMT1L1	2.71E−07	2.83333475	2.83333
TMEM126A	2.96E−08	2.82843025	2.82843
TRF	3.44E−06	2.81864023	2.81864
LOC383189	2.82E−08	2.81376268	2.81376
AW536289	4.33E−07	2.81376268	2.81376
CCNG1	1.58E−05	2.81376268	2.81376

TABLE 1-continued

HAAO	5.90E−06	2.79916809	2.79917
6720467C03RIK	2.29E−11	2.7943264	2.79433
SLC24A3	3.28E−05	2.7948587	2.78949
PSTPIP1	5.32E−06	2.77502588	2.77502
TBX21	4.36E−06	2.76542554	2.76542
PRDX5	7.28E−05	2.76542554	2.76542
EGR3	0.000564399	2.76063119	2.76063
TFF1	0.00864278	2.76063119	2.76063
E130012A19RIK	7.32E−06	2.74631719	2.74632
BCAP29	8.13E−06	2.74631719	2.74632
IRAK2	2.06E−05	2.74156626	2.74157
2310016C08RIK	0.000897768	2.74156626	2.74157
E430036I04RIK	9.77E−08	2.73681675	2.73682
FES	1.41E−07	2.73208368	2.73208
C330023F11RIK	1.27E−07	2.71791568	2.71791
FKBP11	0.000163935	2.7132109	2.71321
GP49A	8.69E−07	2.70850769	2.70851
FHL2	3.43E−08	2.70382077	2.70382
PRMT2	5.08E−07	2.69446637	2.69447
3300005D01RIK	5.96E−05	2.68514764	2.68514
RPS6KA1	5.06E−08	2.66659556	2.6666
GPD2	2.77E−08	2.66198158	2.66198
SNAG1	8.24E−08	2.66198158	2.66198
CLN3	2.04E−05	2.65277321	2.65277
TMPIT	4.29E−07	2.64817685	2.64816
1810011E08RIK	4.07E−06	2.64817885	2.64818
BIN1	6.15E−08	2.64359338	2.64359
PEA15	3.62E−08	2.62988336	2.62989
SDF2L1	4.71E−05	2.62988336	2.62989
LOC383099	3.70E−05	2.62988336	2.62989
4930486L24RIK	2.78E−06	2.60719481	2.6072
CAMK2N1	2.06E−06	2.60268232	2.60266
IFI30	2.03E−06	2.59817868	2.59818
4930513E20RIK	0.000992688	2.59367713	2.59368
0610037M15RIK	7.56E−05	2.58919116	2.58919
PFKP	1.61E−05	2.58470732	2.58471
A630024B12RIK	2.56E−06	2.58023232	2.58023
SPIN2	4.24E−07	2.57576616	2.57576
MMD	1.33E−06	2.57576616	2.57576
MGC18837	1.51E−06	2.56240743	2.56241
C130027E04RIK	3.69E−07	2.55354138	2.55354
IL18R1	5.54E−06	2.55354138	2.55354
GALGT1	1.55E−05	2.55354138	2.55354
STK39	3.36E−08	2.54912157	2.54912
OBFC2A	8.44E−06	2.54912157	2.54912
D930046M13RIK	1.92E−05	2.54471056	2.54471
ALDOC	0.00134083	2.54471056	2.54471
IL2RB	4.01E−07	2.54030189	2.5403
LOC381319	0.000586562	2.53151098	2.53151
BAG3	0.00125353	2.53151098	2.53151
DOK2	1.17E−06	2.52275525	2.52275
UGCG	8.13E−05	2.52275525	2.52275
ARRDC4	0.000487201	2.5183842	2.51839
ATF4	0.00225306	2.5183842	2.51839
IL12RB1	6.73E−07	2.51402828	2.51403
9130211I03RIK	0.000664109	2.51402828	2.51403
1810061M12RIK	7.55E−08	2.50533009	2.50533
KLRA21	1.66E−06	2.50099415	2.50099
MVP	3.59E−07	2.49666072	2.49666
CYBA	1.03E−06	2.49233607	2.49234
BATF	2.83E−06	2.49233607	2.49234
NENF	1.16E−05	2.49233607	2.49234
TNFSF13	2.10E−08	2.48802018	2.48802
EHD4	1.57E−05	2.48802018	2.48802
CSDA	3.96E−08	2.47941466	2.47942
CRELD2	1.86E−05	2.47512499	2.47512
LOC238943	2.92E−06	2.47083794	2.47084
1110019C08RIK	3.97E−05	2.47083794	2.47084
OSTF1	8.10E−08	2.46655962	2.46656
2510048K03RIK	4.28E−07	2.46655962	2.46656
1700025G04RIK	1.15E−05	2.46229003	2.46229
PADI2	1.20E−08	2.45802311	2.45803
A530060O05RIK	9.89E−07	2.45802311	2.45803
FXYD5	9.20E−06	2.45802311	2.45803
MYO1G	6.72E−06	2.45377096	2.45377
ECH1	1.99E−05	2.45377096	2.45377

TABLE 1-continued

GABARAPL1	5.14E−09	2.44528073	2.44528
AI847670	4.70E−07	2.44104867	2.44105
PPIB	3.62E−09	2.43681937	2.43682
FOSL2	1.02E−05	2.42839277	2.42839
BC023892	3.65E−09	2.42418366	2.42419
STX7	1.05E−06	2.42418366	2.42419
NUCB1	1.57E−05	2.42418366	2.42419
KLF7	1.89E−05	2.42418366	2.42419
TAF9B	8.39E−07	2.41998911	2.41999
LOC381683	4.83E−07	2.41998911	2.41999
LOC386405	0.00419371	2.41998911	2.41999
H47	6.51E−07	2.41579738	2.4158
GPR34	1.34E−07	2.41161433	2.41162
1110006I15RIK	1.08E−07	2.40743995	2.40744
MYL6	2.17E−08	2.40327422	2.40327
SOAT2	1.66E−05	2.40327422	2.40327
HIP1	1.04E−10	2.39911137	2.39911
DEGS	5.19E−07	2.39911137	2.39911
2810032E02RIK	1.12E−06	2.39911137	2.39911
SH3BGRL3	0.000169298	2.39911137	2.39911
DTR	4.75E−06	2.38666902	2.38667
SLK	1.18E−08	2.38254074	2.38254
EOMES	8.89E−07	2.3784154	2.37841
GMDS	2.61E−06	2.3784154	2.37841
DCXR	3.22E−05	2.37018497	2.37019
H2-Q8	0.00206753	2.37018497	2.37019
HIP-1	1.62E−10	2.36607988	2.36608
DAB2IP	5.55E−08	2.36607988	2.36608
KLRK1	3.04E−08	2.36607988	2.36608
OLFM1	7.39E−08	2.3619834	2.36199
CABLES1	2.23E−06	2.3619834	2.36199
AI840980	0.000110731	2.3619834	2.36199
BC024955	4.20E−08	2.35789553	2.3579
MINA	4.70E−09	2.35789553	2.3579
A530090P03RIK	6.45E−06	2.35789553	2.3579
NRGN	4.73E−05	2.3538107	2.35381
ZFP52	0.000839487	2.3538107	2.35381
TSPO	2.07E−06	2.34974	2.34974
TDRD7	2.88E−06	2.34567235	2.34567
TMEM38B	6.08E−08	2.34160779	2.34161
SAMSN1	1.16E−06	2.34160779	2.34161
IAN4	0.000116004	2.34160779	2.34161
IMPA2	0.000464842	2.33755184	2.33755
2310056P07RIK	0.000374894	2.33755184	2.33755
ETFB	8.23E−07	2.3335099	2.33351
GZMK	0.000514646	2.32140826	2.32141
5730438N18RIK	1.67E−05	2.31738969	2.31739
LOC215678	4.32E−05	2.31337431	2.31338
LOC269355	1.94E−05	2.30937282	2.30937
STK32C	4.69E−07	2.30537452	2.30537
SLAMF7	1.11E−06	2.30537452	2.30537
ABCB1B	1.12E−06	2.30137945	2.30138
4930539E08RIK	3.24E−05	2.30137945	2.30138
CLNK	3.67E−08	2.2973982	2.2974
TEX9	1.16E−06	2.2973982	2.2974
LOC218617	6.06E−08	2.29342018	2.29342
PALD	8.48E−07	2.29342018	2.29342
GPR114	0.00411637	2.29342018	2.29342
GOLGA7	1.77E−06	2.28945067	2.28945
GPR160	5.41E−06	2.28945067	2.28945
KIT	0.00014555	2.28945067	2.28945
SQSTM1	0.000722465	2.28945067	2.28945
KLRA16	3.18E−07	2.28548443	2.28548
ZBTB32	1.19E−05	2.28548443	2.28548
1110030J09RIK	5.87E−10	2.28152671	2.28153
CYP51	2.62E−06	2.28152671	2.28153
3110054C06RIK	1.87E−06	2.28152671	2.28153
C730026J16	4.19E−05	2.26969929	2.2697
SERTAD1	7.39E−05	2.26969929	2.2697
2310004N11RIK	8.08E−05	2.26969929	2.2697
VEGFC	1.79E−07	2.26576519	2.26577
LOC114601	7.89E−06	2.26576519	2.26577
A930008A22RIK	1.15E−06	2.26184472	2.26184
GFOD1	6.22E−07	2.26184472	2.26184
STK2	7.89E−09	2.25011869	2.25012
BC036961	4.29E−06	2.25011869	2.25012

TABLE 1-continued

1810006K23RIK	4.62E−06	2.25011869	2.25012
2310047C17RIK	0.00113428	2.25011869	2.25012
CAI	2.16E−07	2.23844849	2.23845
CAPNS1	1.49E−08	2.23457196	2.23457
RPL36	3.70E−05	2.23457196	2.23457
2310043N10RIK	7.41E−05	2.23070385	2.23071
SYPL	1.01E−06	2.22684416	2.22684
GZMN	0.00220233	2.22684416	2.22684
COMT	6.85E−09	2.22298792	2.22299
SCL000416.1_19	1.60E−08	2.22298792	2.22299
LOC382127	0.000238522	2.22298792	2.22299
2610009E16RIK	0.000238786	2.21914008	2.21914
GPC1	0.0145298	2.21914008	2.21914
ASAH1	2.95E−08	2.21145978	2.21146
HADH2	7.87E−06	2.21145978	2.21146
XDH	1.37E−06	2.20763223	2.20763
DP1	5.63E−06	2.20380818	2.20381
TGFBR2	3.17E−07	2.19999252	2.19999
SC4MOL	1.44E−06	2.19999252	2.19999
XAB1	1.99E−06	2.19999252	2.19999
PTPN8	3.84E−06	2.19238147	2.19238
DIP3B	3.01E−06	2.18479904	2.1848
VTI1B	8.79E−06	2.18479904	2.1848
STK17B	1.46E−05	2.18479904	2.1848
GNS	0.000892576	2.18479904	2.1848
MTMR9	0.00171537	2.18479904	2.1848
ALAD	2.13E−08	2.18101557	2.18102
ARPC1B	3.71E−07	2.18101557	2.18102
2600010E01RIK	1.17E−05	2.18101557	2.18102
LOC237361	1.67E−05	2.15845662	2.15846
MPP6	2.77E−06	2.15471732	2.15472
PDCD1LG2	8.02E−06	2.15471732	2.15472
SERPINB6B	2.58E−05	2.15471732	2.15472
CAPN2	1.74E−06	2.15099096	2.15099
ELOVL1	2.29E−06	2.15099096	2.15099
RAB3D	2.65E−05	2.15099096	2.15099
HINT2	0.000350321	2.14726363	2.14726
FIGF	4.55E−05	2.1435492	2.14355
OSM	0.000887713	2.1435492	2.14355
ACATE3	1.20E−06	2.13983386	2.13984
5430427O19RIK	3.35E−06	2.13983386	2.13984
EG630499	0.00033688	2.12874206	2.12874
KLK1B11	0.00366553	2.12874206	2.12874
FNBP1	3.25E−08	2.12137669	2.12138
SRI	2.09E−08	2.12137669	2.12138
2610529H08RIK	2.11E−07	2.12137669	2.12138
ANXA5	0.000191309	2.12137669	2.12138
RPIA	2.24E−08	2.11403529	2.11404
LCP1	1.15E−05	2.11403529	2.11404
LOC23352	3.13E−05	2.11037693	2.11038
UGALT2	1.37E−05	2.10672234	2.10672
ANXA3	0.00588432	2.10307596	2.10307
JAM4	2.28E−07	2.09943336	2.09943
CHST12	6.51E−07	2.09943336	2.09943
SCL0001297.1_42	3.97E−05	2.09943336	2.09943
PYGL	9.38E−05	2.09943336	2.09943
MREG	1.19E−07	2.09579897	2.0958
1810003N24RIK	2.62E−06	2.09579897	2.0958
HRC	0.000100933	2.09579897	2.0958
DDIT4	0.0101176	2.09216839	2.09217
FBXO4	2.51E−07	2.08493178	2.08493
201007E07RIK	5.45E−06	2.08493178	2.08493
ZFP296	2.06E−08	2.08132139	2.08132
0610039D01RIK	7.86E−07	2.08132139	2.08132
COTL1	0.000383267	2.08132139	2.08132
KLRI1	1.30E−08	2.07771916	2.07772
UBL4	2.81E−07	2.07771916	2.07772
ARHGAP18	3.33E−07	2.07771916	2.07772
SNX9	1.75E−07	2.07412078	2.07412
PFN1	3.03E−07	2.07412078	2.07412
9130227C08RIK	2.98E−06	2.07412078	2.07412
DAP	2.54E−05	2.07412078	2.07412
9030611O19RIK	7.73E−05	2.07412078	2.07412
D8ERTD354E	0.000213556	2.07412078	2.07412
2900026A02RIK	3.15E−07	2.07053055	2.07053
M6PR	1.54E−06	2.07053055	2.07053

TABLE 1-continued

MRPS6	1.82E−05	2.07053055	2.07053
0610009O03RIK	1.91E−08	2.06694419	2.06695
CAPZB	4.50E−07	2.06336597	2.06337
ARRB2	1.28E−06	2.06336597	2.06337
A430093B03RIK	4.59E−07	2.05979587	2.05979
NIBAN	3.10E−05	2.05979587	2.05979
2610036L11RIK	0.00147553	2.05979587	2.05979
DHRS7	1.18E−08	2.05622966	2.05623
LOC241621	3.38E−06	2.05266734	2.05267
COX7A1	0.000599505	2.05266734	2.05267
RBMS1	2.03E−07	2.04911314	2.04911
CDKN2A	0.000238387	2.04911314	2.04911
BB220380	1.50E−07	2.03849078	2.03849
CMKBR2	3.22E−06	2.03849078	2.03849
AW212394	2.59E−05	2.03849078	2.03849
1110030C22RIK	0.000169536	2.03496062	2.03496
SCL00319622.1__241	8.06E−06	2.03143442	2.03144
LCN4	0.00307845	2.03143442	2.03144
KDELR2	1.28E−06	2.02792041	2.02792
CD59A	0.00082023	2.02440624	2.02441
CAPN5	0.00123881	2.01740211	2.0174
ZFP608	2.50E−06	2.0139121	2.01391
SLC2A6	0.000147228	2.0139121	2.01391
CORO1C	1.61E−06	2.0139121	2.01391
GNPDA1	7.70E−07	2.0139121	2.01391
CARD4	8.05E−06	2.01042607	2.01042
09-Sep	2.79E−06	2.00694403	2.00694
2410012H22RIK	8.81E−07	2.00347001	2.00347
SKAP2	1.02E−06	2.00347001	2.00347
IAN3	0.0043871	2.00347001	2.00347
TPI1	0.00037172	2.00347001	2.00347
9130422G05RIK	8.52E−08	2	2
2810004N20RIK	1.13E−06	2	2
B4GALNT2	0.00262346	2	2
DNMT3B	1.05E−06	0.5	−2
SCL000548.1__6	2.96E−06	0.5	−2
LYT-2	2.32E−05	0.5	−2
LBR	1.18E−05	0.499134	−2.00347
6330406L22RIK	5.22E−05	0.499134	−2.00347
MIER1	1.68E−08	0.498271	−2.00694
1810020D17RIK	2.21E−07	0.498271	−2.00694
SLC9A9	4.02E−05	0.498271	−2.00694
TCRG-V5	0.00106772	0.498271	−2.00694
BC035295	8.94E−08	0.4974085	−2.01042
LOC386192	0.0045284	0.4974085	−2.01042
ARHGEF11	2.19E−08	0.49654652	−2.01391
B230114J08RIK	6.30E−07	0.49654652	−2.01391
ARID1A	2.03E−06	0.49654652	−2.01391
C030002B11RIK	3.75E−05	0.49654652	−2.01391
E430013K19RIK	3.08E−06	0.49568752	−2.0174
A130022A09RIK	4.53E−06	0.49568752	−2.0174
LOC269401	5.58E−06	0.49568752	−2.0174
2700007B13RIK	3.11E−05	0.49568752	−2.0174
DDAH1	0.000139319	0.49568752	−2.0174
HP	0.00181193	0.49568752	−2.0174
DNCHC1	5.02E−06	0.49482904	−2.0209
SPEC1	1.46E−05	0.49397108	−2.02441
KCNH3	5.03E−08	0.49226165	−2.03144
LRMP	3.72E−06	0.49226165	−2.03144
CAMK4	5.35E−08	0.49141015	−2.03496
1110001P04RIK	2.50E−06	0.49141015	−2.03496
COL15A1	7.73E−05	0.49055919	−2.03849
E130307M08RIK	3.42E−05	0.48971117	−2.04202
NFE2	3.74E−05	0.48971117	−2.04202
ASB13	4.76E−07	0.48717037	−2.05267
XLR4A	0.000619052	0.48717037	−2.05267
LOC382020	0.0011986	0.48717037	−2.05267
3110018A08RIK	0.00353392	0.48717037	−2.05267
BC020108	0.000419903	0.48632692	−2.05623
SOX9	0.00119698	0.48632692	−2.05623
CD5	2.42E−06	0.48548638	−2.05979
ZFP96	2.60E−05	0.48548638	−2.05979
AKAP8L	8.62E−07	0.48464405	−2.06337
5530400P07RIK	1.11E−06	0.48380464	−2.06695
A430107D22RIK	2.94E−06	0.48380464	−2.06695
0610012D17RIK	7.81E−08	0.48296813	−2.07053

TABLE 1-continued

GALNT2	5.48E−07	0.48296813	−2.07053
EPPB9	2.51E−05	0.48296813	−2.07053
NSG2	8.56E−08	0.48213218	−2.07412
DUSP10	4.51E−08	0.48129681	−2.07772
9430080K19RIK	3.61E−08	0.48129681	−2.07772
RNASEN	2.84E−06	0.48129681	−2.07772
GAS6	0.000310919	0.48129681	−2.07772
1810015C11RIK	7.75E−09	0.48046432	−2.08132
SLITL2	0.000449972	0.48046432	−2.08132
LOC386330	0.00291956	0.48046432	−2.08132
FKBP9	0.000504748	0.47963241	−2.08493
ZFPN1A1	8.59E−07	0.47880108	−2.08855
DDX6	3.03E−07	0.47797263	−2.09217
BACH1	1.88E−06	0.47797263	−2.09217
TNNT1	0.000226617	0.47797263	−2.09217
BLK	2.09E−08	0.47714477	−2.0958
MSCP	3.61E−07	0.47714477	−2.0958
2900060B14RIK	0.0188022	0.47714477	−2.0958
CNN3	2.16E−06	0.47549535	−2.10307
REEP1	3.13E−08	0.47467153	−2.10672
SDH1	4.36E−08	0.47302795	−2.11404
PPARGC1B	9.21E−07	0.47302795	−2.11404
TLK1	5.15E−07	0.47302795	−2.11404
A630097D09RIK	3.46E−05	0.47221042	−2.1177
3110078M01RIK	8.46E−07	0.47139126	−2.12138
1110015K06RIK	2.05E−06	0.47057716	−2.12505
EXT1	5.14E−06	0.46894857	−2.13243
FBLN2	1.48E−07	0.4681363	−2.13613
1810018P12RIK	4.02E−05	0.46651583	−2.14355
DCAMKL2	1.02E−06	0.46570979	−2.14726
PPT1	1.66E−06	0.46570979	−2.14726
2810036L13RIK	3.90E−05	0.46570979	−2.14726
H2-EB1	0.00495563	0.46490221	−2.15099
2810470K03RIK	6.54E−06	0.46409742	−2.15472
RAG1	2.91E−05	0.46329327	−2.15846
2610020H15RIK	1.83E−07	0.46249191	−2.1622
D10UCLA1	4.00E−05	0.46169117	−2.16595
1110003A17RIK	7.74E−07	0.46089109	−2.16971
KCTD2	2.52E−05	0.46089109	−2.16971
G630024G08RIK	3.72E−08	0.45929709	−2.17724
1700026B20RIK	3.84E−06	0.45850107	−2.18102
FAS	7.68E−09	0.4577078	−2.1848
4933424M23RIK	1.76E−07	0.4577078	−2.1848
4921518A06RIK	4.47E−06	0.4577078	−2.1848
IGTP	0.00151097	0.4577078	−2.1848
9430068D06RIK	6.51E−08	0.45691518	−2.18859
A930005H10RIK	1.37E−07	0.45691518	−2.18859
ABCA3	2.04E−07	0.45533401	−2.19619
5330403D14RIK	3.86E−07	0.45533401	−2.19619
4631427C17RIK	9.36E−07	0.45533401	−2.19619
TRIM28	2.42E−05	0.45454752	−2.19999
CERK	1.15E−05	0.45454752	−2.19999
CRYL1	7.94E−07	0.45375963	−2.20381
IL7R	1.25E−08	0.45297446	−2.20763
IHPK1	4.52E−07	0.45297446	−2.20763
RENBP	1.68E−08	0.45218996	−2.21146
TPST1	1.05E−05	0.44984458	−2.22299
9430029L20RIK	9.20E−09	0.44828776	−2.23071
5730593F17RIK	3.51E−06	0.44828776	−2.23071
C530015C18	1.57E−08	0.4467377	−2.23845
HMGN2	0.00010418	0.4467377	−2.23845
TRAF4	8.39E−08	0.44596469	−2.24233
AXIN2	2.23E−09	0.44519237	−2.24622
BCL7A	5.46E−07	0.44519237	−2.24622
A630082K20RIK	7.65E−08	0.44442074	−2.25012
TNRC6C	1.44E−07	0.44442074	−2.25012
PCOLCE	0.000259954	0.44365179	−2.25402
PRICKLE1	6.89E−07	0.44288353	−2.25793
BCL6	1.24E−05	0.44288353	−2.25793
COL2A1	3.25E−05	0.44211792	−2.26184
MRPL14	5.42E−07	0.44135106	−2.26577
ZFP148	8.82E−07	0.44058686	−2.2697
CNOT2	1.44E−07	0.4398253	−2.27363
C230075L19RIK	1.84E−07	0.43906251	−2.27758
2700083E18RIK	2.52E−07	0.43906251	−2.27758
CCND1	1.92E−05	0.43830237	−2.28153

TABLE 1-continued

CUTL1	8.66E−08	0.43754485	−2.28548
AI467606	5.41E−07	0.43603003	−2.29342
GMFG	7.47E−05	0.43603003	−2.29342
GLDC	0.000564796	0.43603003	−2.29342
CNP1	1.01E−07	0.43527466	−2.2974
RBM38	2.02E−08	0.4345219	−2.30138
BC039093	2.61E−06	0.4345219	−2.30138
6.33E+19	2.02E−05	0.4345219	−2.30138
SCARA3	5.87E−05	0.4345219	−2.30138
FKBP5	3.81E−07	0.43376985	−2.30537
BC063749	1.34E−09	0.43301853	−2.30937
LOC226135	0.000314737	0.43301853	−2.30937
AFF1	5.19E−07	0.43226794	−2.31338
COL4A1	3.11E−05	0.43002799	−2.32543
COL6A3	0.00167802	0.43002799	−2.32543
VAMP4	4.17E−07	0.4292822	−2.32947
NUP210	0.000100262	0.42853898	−2.33351
ADCY6	1.78E−07	0.42779834	−2.33755
UHRF1	0.000151815	0.42779834	−2.33755
PTPRS	3.83E−07	0.4270566	−2.34161
LBH	5.98E−05	0.42631743	−2.34567
SCML4	1.21E−07	0.425579	−2.34974
1700095N21RIK	2.00E−07	0.425579	−2.34974
5930416I19RIK	4.45E−07	0.425579	−2.34974
SEMA4B	1.98E−06	0.425579	−2.34974
SCA2	6.05E−07	0.42484313	−2.35381
5830431A10RIK	2.60E−06	0.42484313	−2.35381
MSH6	0.000132082	0.42484313	−2.35381
TTC3	5.71E−09	0.4219071	−2.37019
KCTD1	1.30E−06	0.4219071	−2.37019
BC028975	1.19E−08	0.42117677	−2.3743
GPSM1	2.16E−06	0.42117677	−2.3743
ERICH1	3.26E−08	0.42044896	−2.37841
GATA3	6.03E−07	0.42044896	−2.37841
TTYH3	4.09E−06	0.42044896	−2.37841
H2-OB	6.36E−06	0.42044896	−2.37841
BHLHB9	1.72E−05	0.42044896	−2.37841
AW046396	0.00133505	0.42044896	−2.37841
4632417D23	5.33E−07	0.41754351	−2.39496
PDLIM1	8.32E−07	0.41754351	−2.39496
1810010N17RIK	1.37E−06	0.41682124	−2.39911
CHRNA9	1.78E−09	0.41609973	−2.40327
GSTM2	0.000400316	0.41537899	−2.40744
SCL000121.1_106	6.07E−06	0.41394155	−2.4158
MNS1	0.000531845	0.41394155	−2.4158
GABABRBP	3.45E−05	0.41250892	−2.42419
ANP32E	3.77E−05	0.41108279	−2.4326
SMARCD2	6.84E−06	0.40402082	−2.47512
CASP6	6.84E−07	0.40332013	−2.47942
LTAP	3.13E−08	0.40053511	−2.49666
EFEMP2	8.18E−05	0.39984166	−2.50099
4932408F19RIK	3.03E−07	0.39845876	−2.50967
CD1D1	9.54E−07	0.39845876	−2.50967
SH2D1A	1.27E−05	0.39845876	−2.50967
ADRB2	1.53E−08	0.39776773	−2.51403
6330403E01RIK	2.81E−07	0.39707909	−2.51839
C230082I21RIK	1.32E−09	0.39639283	−2.52275
FBXL12	3.35E−06	0.39502115	−2.53151
SMO	3.82E−09	0.39433732	−2.5359
6720469N11RIK	1.55E−07	0.39365429	−2.5403
ZFPN1A2	1.20E−07	0.39297209	−2.54471
PHF2	2.68E−07	0.39297209	−2.54471
LIP1	8.34E−07	0.39297209	−2.54471
IFNGR1	1.21E−07	0.39229224	−2.54912
SPATA13	1.64E−07	0.39161321	−2.55354
NEDD4L	4.00E−09	0.390935	−2.55797
SLA	4.00E−05	0.390935	−2.55797
ARHGEF18	2.56E−05	0.390935	−2.55797
RASGRP1	7.85E−08	0.39025761	−2.56241
NOTCH1	2.02E−08	0.38823493	−2.57576
2900016B01RIK	6.34E−09	0.38756235	−2.58023
PITPNM2	1.79E−07	0.3868906	−2.58471
SMAD3	2.00E−06	0.38555257	−2.59368
CHDH	1.11E−07	0.38355324	−2.6072
C920011N12RIK	5.34E−06	0.38355324	−2.6072
NISCH	5.43E−07	0.38288944	−2.61172

TABLE 1-continued

2310007G05RIK	1.06E−06	0.38288944	−2.61172
SIT1	3.76E−09	0.38222647	−2.61625
SLC29A3	7.22E−07	0.3809045	−2.62533
AEBP1	0.000162054	0.3809045	−2.62533
C730009F21RIK	9.48E−09	0.37958587	−2.63445
PLEKHG2	9.65E−07	0.37958587	−2.63445
MBP	2.65E−08	0.37827348	−2.64359
D8ERTD325E	9.64E−06	0.37827348	−2.64359
VPS54	4.16E−08	0.37761784	−2.64818
MLL	2.80E−07	0.37761784	−2.64818
LOC386144	4.21E−05	0.37761784	−2.64818
LOC386360	0.000403683	0.37761784	−2.64818
BACH2	0.000136247	0.37696446	−2.65277
BDH	2.28E−10	0.37500938	−2.6666
KLHL6	2.22E−08	0.37500938	−2.6666
DAP3	1.93E−08	0.37371163	−2.67586
TCRB-V8.2	8.04E−08	0.37371163	−2.67586
A130062D16RIK	1.07E−07	0.37371163	−2.67586
SSBP3	1.03E−07	0.37371163	−2.67586
MAPK1	5.13E−08	0.37242006	−2.68514
FRMD6	3.31E−08	0.37177485	−2.6898
TNFRSF13B	6.85E−08	0.37177485	−2.6898
MMP2	0.000109136	0.37177485	−2.6898
ECM1	1.84E−07	0.3711305	−2.69447
CUL7	2.80E−07	0.36602149	−2.73208
NOTCH3	7.70E−06	0.36602149	−2.73208
D930015E06RIK	2.19E−07	0.36538757	−2.73682
A430106G13RIK	1.00E−06	0.3647545	−2.74157
5830468F06RIK	5.09E−06	0.36412363	−2.74632
HIBADH	1.23E−08	0.36349361	−2.75108
TMEM9	4.73E−07	0.36223616	−2.76063
TSPAN32	8.74E−07	0.35662957	−2.80403
H2-T9	4.22E−05	0.35662957	−2.80403
ESM1	0.00132143	0.35601252	−2.80889
ALOX5AP	1.28E−07	0.35539634	−2.81376
RFX2	1.13E−06	0.35539634	−2.81376
2610019F03RIK	3.33E−06	0.35478103	−2.81864
WHRN	4.89E−07	0.35355303	−2.82843
5830496L11RIK	1.90E−07	0.35294159	−2.83333
GSTP1	7.09E−07	0.35294159	−2.83333
3100002J23RIK	3.91E−07	0.35232978	−2.83825
YPEL3	0.0001326	0.35111127	−2.8481
A130092J06RIK	3.76E−06	0.35050332	−2.85304
IGSF3	5.35E−07	0.34929007	−2.86295
HDAC7A	1.07E−06	0.34448192	−2.90291
IDB3	1.63E−07	0.34388605	−2.90794
OLFML3	6.69E−07	0.34328988	−2.91299
6430510M02RIK	1.07E−07	0.34269578	−2.91804
TRBV13-1__M15618__T__CELL__RECEPTOR__BETA__VARIABLE__13-	1.50E−05	0.34151023	−2.92817
CD97	2.26E−05	0.33973969	−2.94343
MTF2	1.12E−06	0.33797714	−2.95878
PLA2G12A	1.72E−08	0.33622487	−2.9742
D15WSU75E	2.04E−06	0.33622487	−2.9742
ETHE1	1.66E−08	0.33448172	−2.9897
HIVEP3	3.41E−09	0.33390319	−2.99488
CYB5	3.77E−08	0.33390319	−2.99488
CTSE	0.149884	0.33332444	−3.00008
ZFP219	2.26E−07	0.32987732	−3.03143
ABHD8	1.69E−06	0.32987732	−3.03143
4732481H14RIK	8.63E−07	0.32930592	−3.03669
PRNP	3.14E−05	0.32873542	−3.04196
A630038E17RIK	0.000216397	0.32873542	−3.04196
A930013B10RIK	8.09E−08	0.3253355	−3.07375
ETS1	5.90E−07	0.32252862	−3.1005
LOC385086	0.00104356	0.32252862	−3.1005
RNPEPL1	7.60E−09	0.32196994	−3.10588
KLF13	6.51E−07	0.32141319	−3.11126
KCNN4	1.98E−06	0.3208563	−3.11666
NIPSNAP1	1.39E−06	0.3208563	−3.11666
C920004C08RIK	0.00056251	0.3208563	−3.11666
TRBV7__AE000663__T__CELL__RECEPTOR__BETA__VARIABLE__7__29	6.95E−05	0.31974625	−3.12748
SLC43A1	3.64E−08	0.31643867	−3.16017
STK4	7.89E−07	0.31589089	−3.16565
WISP2	1.27E−05	0.31262407	−3.19873
ACTN2	3.36E−07	0.31046452	−3.22098

TABLE 1-continued

EPB4.1L4B	4.42E−07	0.31046452	−3.22098
RNF144	2.74E−09	0.30885455	−3.23777
SCL0001849.1_2273	1.73E−05	0.30778606	−3.24901
ART4	6.83E−07	0.30619056	−3.26594
18S_RRNA_X00686_301	0.00445027	0.29988874	−3.33457
A330103N21RIK	1.65E−06	0.29936983	−3.34035
TPCN1	2.60E−09	0.29885092	−3.34615
AJ237586	1.97E−07	0.29833381	−3.35195
BC026370	6.26E−09	0.29575998	−3.38112
EPHX1	4.14E−09	0.29524827	−3.38698
COL5A1	0.000124246	0.29371767	−3.40463
SNN	1.47E−08	0.29270149	−3.41645
BCL9L	1.00E−08	0.29219432	−3.42238
0710008K08RIK	9.65E−08	0.29168806	−3.42832
F730003H07RIK	0.000110195	0.28967371	−3.45216
AI504432	2.49E−08	0.28717477	−3.4822
OACT1	9.74E−09	0.28667752	−3.48824
A130093I21RIK	1.16E−06	0.28519116	−3.50642
RGS10	2.26E−05	0.28469751	−3.5125
1110046J11RIK	3.46E−06	0.28420394	−3.5186
E2F2	2.29E−06	0.27835537	−3.59253
BRD3	3.58E−07	0.27787349	−3.59876
AA408556	4.12E−06	0.27262665	−3.66802
SATB1	1.49E−07	0.27074371	−3.69353
ILVBL	3.59E−07	0.27074371	−3.69353
TIAM1	1.97E−09	0.26887358	−3.71922
POU6F1	2.59E−08	0.26887358	−3.71922
MAGED1	1.17E−05	0.26887358	−3.71922
1810055G02RIK	1.69E−09	0.26655436	−3.75158
0710001E13RIK	6.72E−10	0.26471273	−3.77768
LMAN2L	4.14E−08	0.26471273	−3.77768
SLC29A1	1.20E−07	0.26425455	−3.78423
3830612M24	2.98E−07	0.26379726	−3.79079
H2-T10	0.000216723	0.26379726	−3.79079
CXCR4	4.41E−08	0.26334015	−3.79737
RIL-PENDING	2.35E−08	0.26242931	−3.81055
SERPINH1	3.41E−05	0.26242931	−3.81055
PALM	2.59E−09	0.26197487	−3.81716
CXCL12	0.000158114	0.26106866	−3.83041
5430417L22RIK	1.68E−08	0.2606162	−3.83706
TRIB2	4.82E−08	0.26016531	−3.84371
ETS2	5.78E−08	0.26016531	−3.84371
ALDH2	9.48E−07	0.25881592	−3.86375
HMGN1	3.20E−05	0.25702844	−3.89062
TRP53INP1	4.08E−08	0.25569562	−3.9109
ITPR2	5.70E−08	0.25392956	−3.9381
TCRB	8.36E−06	0.25348992	−3.94493
A930023F05RIK	4.58E−09	0.24784315	−4.03481
SLC5A9	1.20E−06	0.24698554	−4.04882
ICAM2	2.53E−07	0.24655805	−4.05584
H2-DMA	1.25E−05	0.24655805	−4.05584
4932414K18RIK	3.75E−08	0.24443125	−4.09113
TAP2	9.85E−09	0.24358518	−4.10534
TRBV12-2_M15613_T_CELL_RECEPTOR_BETA_VARIABLE_12-	3.25E−05	0.24358518	−4.10534
PRELP	8.99E−06	0.24232261	−4.12673
TRBV12-1_M15614_T_CELL_RECEPTOR_BETA_VARIABLE_12-	1.47E−06	0.24106609	−4.14824
LOX	8.44E−06	0.24023216	−4.16264
1500004A08RIK	1.03E−07	0.2398162	−4.16986
6720418B01RIK	4.27E−08	0.23857238	−4.1916
4930572J05RIK	4.75E−07	0.23692472	−4.22075
SCL0001032.1_178	7.57E−06	0.23692472	−4.22075
PSAP	4.23E−08	0.23610521	−4.2354
ASS1	5.82E−08	0.23569619	−4.24275
PARD6G	1.30E−08	0.23528803	−4.25011
1500009L16RIK	9.73E−07	0.2324511	−4.30198
GM2A	2.89E−06	0.23124595	−4.3244
LAT	5.46E−08	0.23084559	−4.3319
C3	9.90E−06	0.23084559	−4.3319
PPAP2B	1.69E−05	0.23044607	−4.33941
CTLA4	5.06E−06	0.23044607	−4.33941
FBP1	5.98E−05	0.22845811	−4.37717
B3BP	1.28E−10	0.22453292	−4.45369
PRKCB	6.59E−08	0.22453292	−4.45369
PPP1R1C	3.85E−09	0.22067553	−4.53154

TABLE 1-continued

RAPGEF3	6.21E−10	0.21953174	−4.55515
BAMBI-PS1	7.87E−08	0.21915118	−4.56306
1700012H17RIK	5.26E−09	0.21839306	−4.5789
ACVR2B	3.12E−10	0.21801502	−4.58684
18S_RRNA_X00686_849	0.000602805	0.21538634	−4.64282
SERPINF1	1.43E−05	0.21464156	−4.65893
NAV1	4.40E−08	0.21426995	−4.66701
TBXA2R	3.11E−08	0.2135283	−4.68322
SCL0001132.1_96	1.63E−05	0.20732994	−4.82323
SOX4	1.93E−09	0.2055414	−4.8652
E430021E22RIK	3.57E−07	0.20447556	−4.89056
LOC381739	2.02E−08	0.203063	−4.92458
CD6	3.02E−10	0.20096261	−4.97605
H2-OA	1.09E−07	0.19888426	−5.02805
LOC384370	7.41E−06	0.1978529	−5.05426
ZDHHC8	3.73E−09	0.19751019	−5.06303
AI481316	1.75E−09	0.19682715	−5.0806
H2-BL	3.80E−06	0.19614612	−5.09824
AA407270	7.17E−09	0.1954675	−5.11594
ITGAE	6.39E−06	0.1951288	−5.12482
GPR83	1.37E−07	0.19479128	−5.1337
SBK	4.54E−10	0.18783011	−5.32396
RPS6KL1	1.75E−09	0.18750504	−5.33319
TCF7	8.08E−08	0.18396455	−5.43583
NRP	4.24E−08	0.18364596	−5.44526
DNAJC6	3.58E−11	0.18269378	−5.47364
SCL0001090.1_202	3.66E−09	0.18269378	−5.47364
SCL0001131.1_227	1.31E−06	0.17924456	−5.57897
IL17RB	1.94E−09	0.17800626	−5.61778
ACAS2L	2.63E−07	0.17647048	−5.66667
AKR1C12	8.40E−09	0.1761652	−5.67649
COL6A1	3.56E−05	0.17464534	−5.72589
SOCS3	3.74E−08	0.17283904	−5.78573
LDH2	1.01E−07	0.17283904	−5.78573
DGKA	6.54E−07	0.17283904	−5.78573
GM525	1.28E−07	0.17253963	−5.79577
TIMP2	2.00E−08	0.17224096	−5.80582
AQP11	3.40E−09	0.17045939	−5.8665
TRBV6_AE000663_T_CELL_RECEPTOR_BETA_VARIABLE_6_11	5.66E−08	0.16695132	−5.98977
TNFRSF7	9.20E−08	0.1615441	−6.19026
CD2	7.22E−08	0.15932015	−6.27667
DTX1	3.94E−09	0.15876897	−6.29846
AI875142	2.49E−07	0.15712672	−6.36429
IGFBP4	1.57E−08	0.15577125	−6.41967
SH3KBP1	2.13E−10	0.15496356	−6.45313
2510015F01RIK	3.11E−06	0.15362659	−6.50929
TRBV11_AE000663_T_CELL_RECEPTOR_BETA_VARIABLE_11_	2.50E−08	0.15151148	−6.60016
SLC16A5	1.12E−10	0.14865092	−6.72717
TUBB2B	4.82E−08	0.14813652	−6.75053
DNTT	7.39E−07	0.14533954	−6.88044
SYTL1	6.70E−08	0.14309058	−6.98858
2410008J05RIK	2.59E−09	0.13726158	−7.28536
0610041G09RIK	9.40E−06	0.13702403	−7.29799
2210408F11RIK	2.70E−08	0.13513751	−7.39987
LOC386545	2.77E−05	0.13121465	−7.6211
TRBV31_X03277_T_CELL_RECEPTOR_BETA_VARIABLE_31_33	4.97E−06	0.12918411	−7.74089
TCRB-V8.3	1.15E−08	0.12829181	−7.79473
KLF2	3.06E−06	0.125434	−7.97232
TCRB-V13	5.74E−08	0.12435182	−8.0417
A130038J17RIK	8.77E−09	0.11784809	−8.4855
LOC381738	1.87E−09	0.11723701	−8.52973
G22P1	5.44E−10	0.11582345	−8.63383
CD27	4.55E−08	0.11383376	−8.78474
TMEM108	8.61E−08	0.11265625	−8.87656
ACTN1	2.20E−09	0.11091295	−9.01608
ST6GAL1	6.69E−10	0.10657936	−9.38268
9626100_15	1.11E−05	0.10348549	−9.66319
9626100_224	8.13E−06	0.10118027	−9.88335
C030046M14RIK	1.45E−12	0.1010051	−9.90049
SELL	6.85E−09	0.09944213	−10.0561
COX6A2	7.37E−05	0.0987547	−10.1261
FRAT2	6.49E−09	0.09841357	−10.1612
LY6D	1.48E−09	0.09790292	−10.2142
9130430L19RIK	2.71E−10	0.09278245	−10.7779
CDCA7	3.37E−07	0.09214212	−10.8528
LOC382896	3.09E−09	0.09150554	−10.9283

TABLE 1-continued

CD8B	7.37E−08	0.08993372	−11.1193
E430002D04RIK	2.09E−10	0.08931203	−11.1967
TRGV2__M12831__T__CELL__RECEPTOR__GAMMA__VARIABLE__2__3	2.57E−11	0.08656959	−11.5514
PP11R	2.12E−07	0.08641922	−11.5715
CD81	4.58E−08	0.08318706	−12.0211
IGH-6	8.08E−09	0.08289881	−12.0629
AI132321	2.09E−08	0.08204186	−12.1889
9626958__317	8.03E−06	0.07721352	−12.9511
TRBV8__AE000663__T__CELL__RECEPTOR__BETA__VARIABLE__8__27	9.31E−08	0.07694675	−12.996
MGST2	3.44E−09	0.07419664	−13.4777
RAMP1	5.23E−08	0.072043	−13.8806
NCK2	3.02E−08	0.06863088	−14.5707
MARCKS	1.18E−09	0.06572116	−15.2158
DPP4	2.19E−10	0.05642577	−17.7224
TRBV1__AE000663__T__CELL__RECEPTOR__BETA__VARIABLE__1__20	6.96E−07	0.05603622	−17.8456
H19	3.39E−07	0.0557454	−17.9387
TCRG-V4	3.97E−09	0.05129626	−19.4946
1190002H23RIK	4.58E−10	0.05024065	−19.9042
BCL11B	2.77E−10	0.0495491	−20.182
CD3G	8.20E−11	0.04647186	−21.5184
BGN	3.71E−07	0.03969215	−25.1939
CD3D	1.53E−09	0.0349758	−28.5912
CD3E	7.19E−10	0.03190668	−31.3414
LOC434197	5.40E−11	0.02356029	−42.4443
MYLC2PL	8.10E−10	0.01703917	−58.6883
PDLIM4	8.76E−11	0.00978646	−102.182

TABLE 2

Comparison of cell surface receptor repertoires of ITNKs and LAKs.

Cell Type	Ly49C/I	Ly49D	Ly49G2	NK1.1	NKp46	NKG2A/C/E	NKG2D	CD3
DN3-reprogrammed ITNK (in vitro)	−	−	−	+	+	+	−	−
DP-reprogrammed ITNK (in vitro)	−	−	−	+	+	+	ND	+
DP-reprogrammed ITNK (in vivo)	+	−	+	+	+	+	+	low
LAK	+	+	+	+	+	+	+	−

Note:

N.D., not determined. +, present;. −, absent; low, low levels.

TABLE 3

Column ID	Fold-Change (+OHT vs. −OHT)
24 hours +OHT vs. −OHT	
TCRB-V13	−14894.3
TCRB-V13	−412.694
HIST1H2AO	−35.3085
MTDNA__CYTB	−13.7646
IFITM1	−12.6519
CDCA7	−12.2615
PDLIM4	−10.206
CD3D	−9.81142
RPS29	−9.27924
MTDNA__COXIII	−8.38116
RPS14	−6.99771
MYLC2PL	−6.76599
CD3D	−5.74896
IFITM2	−5.49522
RPL13	−5.33629
RPS17	−5.30823
RPL41	−5.29871
18S__RRNA__X00686__301	−5.08974

TABLE 3-continued

Column ID	Fold-Change (+OHT vs. −OHT)
HIST2H2AC	−4.87534
IFITM3	−4.86541
MTDNA_ND4	−4.61459
HIST1H2AI	−4.60413
MT-CYTB	−4.34
MYLC2PL	−4.32543
RPS11	−4.20813
ITGB7	−4.05052
RPL39	−3.69839
RPS27L	−3.63035
CD3G	−3.49152
EG668668	−3.46858
CD160	−3.44386
RPL23	−3.38327
CD3E	−3.30996
HIST1H2AO	−3.29719
EMP3	−3.22525
PDLIM4	−3.15574
TBCA	−3.1281
THY1	−3.11285
CD8B	−3.01933
PRKACB	−2.90749
HIST1H2AF	−2.78505
LOC226574	−2.74888
G22P1	−2.74634
HIST1H2AG	−2.64205
AI481316	−2.62333
IGH-6	−2.5636
A130092J06RIK	−2.55682
TCRG-V4	−2.54552
UBB	−2.45105
LOC434197	−2.4265
RPS17	−2.41723
MTDNA_ATP6	−2.41506
TXNIP	−2.40294
LOC381808	−2.39227
PPIA	−2.37637
LOC382896	−2.35265
HMGCS1	−2.30359
TCRB-V8.2	−2.2788
HMGN2	−2.2528
UPP1	−2.22963
CSTB	−2.21395
PSAP	−2.20351
TRBV1_AE000663_T_CELL_RECEPTOR_BETA_VARIABLE_1_207	−2.17733
HIBADH	−2.16873
E430002D04RIK	−2.15383
CDCA7	−2.15035
RPS27	−2.14844
RPL8	−2.11346
RNPEPL1	−2.11344
COX6A2	−2.10037
CD27	−2.07584
MARCKS	−2.05494
VIM	−2.04688
AA408556	−2.03945
4932414K18RIK	−2.01727
BCL11B	−2.011
RPS14	−2.0086
TCRB-V8.2	−2.00629
COX7C	2.11831
LOC270037	2.16383
RPA1	2.26914
LAPTM5	2.8707
UBL5	3.35851
ATP5G3	3.54832

TABLE 3-continued

Column ID	Fold-Change (+OHT vs. −OHT)
1300002F13RIK	3.6466
CD52	3.81745
LDH1	3.82439
CYBA	6.431
FCER1G	7.97969
HMGCS1	27.1719
48 hours +OHT vs. −OHT	
ROG	11.7941537
FCER1G	11.6317801
UPP1	9.00046788
IFITM1	8.6938789
SCIN	8.6938789
SERPINA3G	8.51496146
XCL1	7.62110398
AQP9	7.4127045
NKG7	7.01284577
IFITM2	6.40855902
IFITM3	6.36429187
9130404D14RIK	5.69620078
GADD45G	5.6177795
LGALS3	5.46416103
CD160	5.31474326
KLRD1	5.27803164
VIM	4.9933222
TYROBP	4.89056111
LITAF	4.82323131
BC025206	4.78991482
AVIL	4.72397065
LMNA	4.72397065
GLRX1	4.40762046
NFIL3	4.40762046
LTA	4.1410597
CCR5	4.0278222
WBSCR5	4
P2RY14	3.91768119
1300002F13RIK	3.83705648
AMICA1	3.73213197
LOC270152	3.70635225
9130211I03RIK	3.6553258
CDKN2B	3.6553258
PLCG2	3.55537072
CTSW	3.53081199
BC049975	3.50642289
LOC381140	3.36358566
LGALS1	3.34035168
MT1	3.27160823
SYTL2	3.27160823
GPR114	3.24900959
S100A1	3.24900959
2310067E08RIK	3.20427951
LRRK1	3.20427951
TNFRSF11B	3.18214594
IDB2	3.16016525
CCL4	3.11665832
E030006K04RIK	3.11665832
OSBPL3	3.11665832
LY6A	3.09512999
TNFRSF9	3.09512999
S100A6	3.07375036
1500031H04RIK	3.05251842
2210411K11RIK	3.05251842
CTNNA1	3.03143313
LOC381319	3.03143313
EMILIN2	3.01049349
1110018K11RIK	2.9896985
ANXA2	2.9896985
SIAT10	2.96904714
2310046K01RIK	2.94853843
CISH	2.92817139
1110004P15RIK	2.90794503
GOLPH2	2.88785839
HAVCR2	2.88785839

TABLE 3-continued

Column ID	Fold-Change (+OHT vs. −OHT)
PLSCR1	2.88785839
SLC2A6	2.8679105
CAPG	2.84810039
LAG3	2.84810039
F2R	2.82842712
LOC269941	2.82842712
1190002C06RIK	2.80888975
CD9	2.78948733
S100A11	2.78948733
GCNT1	2.75108364
CDKN1A	2.73208051
KLRE1	2.73208051
GPC1	2.71320865
SERPINE2	2.69446715
LRP12	2.67585511
MLKL	2.67585511
BC024955	2.65737163
BHLHB2	2.65737163
C330008K14RIK	2.65737163
F2RL2	2.63901582
GLRX	2.63901582
IFNG	2.62078681
PGLYRP1	2.62078681
1110007C02RIK	2.60268371
BC029169	2.60268371
TRAF1	2.60268371
CDKN2A	2.58470566
DUSP6	2.58470566
LY6G5B	2.58470566
RGS1	2.5668518
MYO1F	2.54912125
HBA-A1	2.53151319
2310047C17RIK	2.51402675
AIM1L	2.51402675
PILRB	2.4966611
2410008K03RIK	2.4794154
APOB48R	2.4794154
PDGFA	2.4794154
FURIN	2.46228883
SPP1	2.46228883
ROM1	2.44528056
SH3BP2	2.44528056
PPP3CC	2.42838977
B4GALNT4	2.41161566
IER3	2.41161566
OSM	2.41161566
DAPK2	2.39495741
LOC218482	2.39495741
MAPKAPK3	2.39495741
PLP2	2.37841423
BAG3	2.36198532
OSTF1	2.36198532
SERPINB6A	2.3456699
FXYD4	2.32946717
LOC327957	2.32946717
AHNAK	2.29739671
CD69	2.28152743
HK2	2.28152743
FES	2.26576777
IL18R1	2.26576777
PPAP2C	2.26576777
SLC39A4	2.25011697
TES	2.25011697
TNF	2.25011697
HGFAC	2.23457428
CD244	2.21913894
6330414G02RIK	2.20381023
CD63	2.20381023
LOC383981	2.1885874
NAPSA	2.1885874
PKP3	2.1885874
EMP1	2.17346973
FOSL2	2.17346973

TABLE 3-continued

Column ID	Fold-Change (+OHT vs. −OHT)
GLIPR1	2.17346973
NT5E	2.17346973
SLC24A3	2.17346973
2610009E16RIK	2.15845647
1110020C13RIK	2.14354693
D10BWG1379E	2.14354693
ID2	2.14354693
DOK2	2.12874036
LOC381924	2.12874036
2210008N01RIK	2.11403608
5330403J18RIK	2.11403608
HIST1H1C	2.09943337
0610037M15RIK	2.08493152
7420404O03RIK	2.08493152
A430006M23RIK	2.07052985
D930046M13RIK	2.07052985
GNG2	2.07052985
GPR68	2.07052985
H2-Q8	2.07052985
IFI30	2.07052985
ZFP608	2.07052985
DCI	2.05622765
NFKB1	2.05622765
PIM3	2.05622765
SGK	2.05622765
CCNG1	2.04202425
CYP51	2.04202425
LOC385953	2.04202425
EGR1	2.02791896
HHEX	2.02791896
MYO1E	2.02791896
TMEM126A	2.02791896
NCF4	2.0139111
PDLIM7	2.0139111
CXCL9	2
GPR18	2
MVP	2
PRSS19	2
A130038J17RIK	−2.0139111
A130093I21RIK	−2.0139111
EPHX1	−2.0139111
NOTCH3	−2.0139111
MTF2	−2.027919
TNFRSF7	−2.027919
4932414K18RIK	−2.0420243
GFI1	−2.0420243
2410008J05RIK	−2.0562277
2610019F03RIK	−2.0705298
H2-OB	−2.0705298
SATB1	−2.0705298
TCF7	−2.0705298
2900060B14RIK	−2.0849315
TBXA2R	−2.0849315
NISCH	−2.0994334
LOC434197	−2.1140361
PARD6G	−2.1140361
DPP4	−2.1435469
H2-AB1	−2.1435469
LMAN2L	−2.1435469
BRD3	−2.1584565
CD27	−2.1584565
LOC386192	−2.1584565
H2-EB1	−2.1734697
NCK2	−2.1734697
RAMP1	−2.1734697
1110046J11RIK	−2.1885874
AQP11	−2.2345743
SLA	−2.2345743
MARCKS	−2.250117
IGH-6	−2.2657678
SH2D1A	−2.2657678
F730003H07RIK	−2.2973967
H2-T10	−2.2973967

TABLE 3-continued

Column ID	Fold-Change (+OHT vs. −OHT)
DGKA	−2.3133764
DNTT	−2.3133764
ETS1	−2.3294672
LOC268393	−2.3294672
LOC386360	−2.3294672
TMEM108	−2.3294672
C230098O21RIK	−2.3619853
RNPEPL1	−2.3619853
G22P1	−2.3784142
TRBV31_X03277_T_CELL_RECEPTOR_BETA_VARIABLE_31_33	−2.3784142
ALDH2	−2.4283898
CDCA7	−2.4622888
NRP	−2.4622888
TXNIP	−2.4622888
SLC16A5	−2.4966611
ACAS2L	−2.5140267
FRAT2	−2.5491213
CD81	−2.6390158
PRKCB	−2.6573716
PDLIM4	−2.6758551
H2-BL	−2.7132087
PP11R	−2.7320805
ACTN1	−2.7510836
CD6	−2.7510836
CD2	−2.7894873
ST6GAL1	−2.8088898
TRBV1_AE000663_T_CELL_RECEPTOR_BETA_VARIABLE_1_20	−2.8088898
CD8B	−2.8481004
9430068D06RIK	−2.8679105
AI132321	−3.0737504
H19	−3.1601652
LY6D	−3.3869812
CTSE	−3.5064229
BCL11B	−3.5801003
LOC382896	−4.5947934
COX6A2	−6.2766728

TABLE 4

The list of primers in this study.

Genotyping PCR primers	Primer sequences (5'-3')	SEQ ID NO.	Size of PCR products (bp)
Genotyping primers.			
Bcl11b-cko-FW	TGAGTCAATAAACCTGGGCGAC	1	243 (wild type);
Bcl11b-cko-RV	GGAATCCTTGGAGTCACTTGTGC	2	345 (flox);
Bcl11b-cko-DEL	TCCTGGTAACACACAATTGC	3	450 (del)
qPCR primers	Primer sequences (5'-3')	SEQ ID NO.	
qRT-PCR primers.			
Notch1-Fwd	CCCTTGCTCTGCCTAACGC	4	
Notch1-Rev	GGAGTCCTGGCATCGTTGG	5	
Etsi-Fwd	TTAGGAAAGGCTCGTTTGCTC	6	
Ets1-Rev	CCAAAGCACAAGCATAGTTTGC	7	
Hes1-Fwd	CCAGCCAGTGTCAACACGA	8	
Hes1-Rev	AATGCCGGGAGCTATCTTTCT	9	
Gata3-Fwd	CTCGGCCATTCGTACATGGAA	10	

TABLE 4-continued

The list of primers in this study.		
Gata3-Rev	GGATACCTCTGCACCGTAGC	11
Deltax1-Fwd	TGTTCAGGCTATACACGCATCAA	12
Deltax1-Rev	CCACCGCCCACTTTCAAG	13
Tcf1-Fwd	ATGGGCGGCAACTCTTTGAT	14
Tcf1-Rev	CGTAGCCGGGCTGATTCAT	15
Cdkn1c-Fwd	CGAGGAGCAGGACGAGAATC	16
Cdkn1c-Rev	GAAGAAGTCGTTCGCATTGGC	17
Id2-Fwd	ATGAAAGCCTTCAGTCCGGTG	18
Id2-Rev	AGCAGACTCATCGGGTCGT	19
Il2rb-Fwd	TGGAGCCTGTCCCTCTACG	20
Il2rb-Rev	TCCACATGCAAGAGACATTGG	21
Zfp105-Fwd	GGCATCCAGCCAACAAGTGTA	22
Zfp105-Rev	CATTTCCTGACCCTTTTCCTCAT	23
Traf1-Fwd	GGAGGCATCCTTTGATGGT A	24
Traf1-Rev	AGGGACAGGTGGGTCTTCTT	25
Zbtb32-Fwd	GCTCTGAGAGAGGACTTGGGA	26
Zbtb32-Rev	TGCTTTATGCTTGTGTGACATCT	27
PCR primers	Primer sequences (5'-3')	SEQ ID NO.
Tcrb rearrangement PCR primers.		
TCRB_Dβ2-Fwd	GTAGGCACCTGTGGGGAAGAAACT	28
TCRB_Vβ2-Fwd	GGGTCACTGATACGGAGCTG	29
TCRB_Jβ2-Rev	TGAGAGCTGTCTCCTACTATCGATT	30
List of primers for ChIP assay qPCR.		
BS1-Fwd	CCGCTACGAGGCACCCTCCTTT	31
BS1-Rev	AGTCTCCTTGGGAAGCACGCGCTA	32
Bs2-Fwd	GCTTGCTTGTTTTTAATTCAGTTTATGGG	33
BS2-Rev	TTGAATGTCTGTGTTGGTGTGTAATCAC	34
BS3-Fwd	GTGAAAAAAAGGGGGTAGGCCCTC	35
BS3-Rev	CAGCCCAAAGTCAAAAGGCAAGATG	36
CTL-Fwd	GTTCCTTAACTGAGAGTTCCTCCTCCC	37
CTL-Rev	TCACTCTGGGCCGGAGTCAGTT	38

SEQUENCE LISTING

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<223> OTHER INFORMATION: CTL-Rev qPCR primer for ChIP assay

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1-45. (canceled)

46. A method of producing induced T-to-Natural-Killer [ITNK] cells from T cells and/or pro-T cells, the method comprising modulating the activity and/or effect of at least one Bcl11b gene and/or protein present in a T cell and/or pro-T cell, and converting said T cell and/or pro-T cell to an ITNK cell or cells.

47. A method of producing target T cells and/or target pro-T cells, the method comprising modulating the activity and/or effect of at least one BcI11b gene and/or protein product present in a T cell and/or pro-T cell, and converting said T cell and/or pro-T cell to said target T cells and/or target pro-T cells.

48. A method according to claim **46** wherein said modulating of the activity and/or effect of said Bcl11b gene and/or protein product comprises inhibiting said activity and/or effect.

49. A method according to claim **47** wherein said modulating of the activity and/or effect of said Bcl11b gene and/or protein product comprises inhibiting said activity and/or effect.

50. A method according to claim **46**, wherein said inhibiting of the activity and/or effect of said Bcl11b gene and/or protein product comprises deletion of at least part of said Bcl11b gene.

51. A method according to claim **47**, wherein said inhibiting of the activity and/or effect of said Bcl11b gene and/or protein product comprises deletion of at least part of said Bcl11b gene.

52. A method according to claim **46**, wherein said modulating of the activity and/or effect of said Bcl11b gene and/or protein product comprises directly or indirectly modulating the activity and/or effect of said Bcl11b protein.

53. A method according to claim **47**, wherein said modulating of the activity and/or effect of said Bcl11b gene and/or protein product comprises directly or indirectly modulating the activity and/or effect of said Bcl11b protein.

54. A method according to claim **46**, which comprises directly or indirectly inhibiting the activity and/or effect of said Bcl11b protein.

55. A method according to claim **47**, which comprises directly or indirectly inhibiting the activity and/or effect of said Bcl11b protein.

56. An isolated ITNK cell characterized by exhibiting one or more or all of the following properties:

(a) a morphology comparable to natural killer cells, in comparison to T cells;

(b) TCR β specific genomic DNA re-arrangement;

(c) a gene expression profile more similar to that of NK cells than the parental cells from which they were developed;

(d) cellular expression of one or more NK specific genes;

(e) decreased or no expression of one or more T lineage genes, in comparison to the parent cells from which the ITNK cell was derived;

(f) cell killing ability; and

(g) capable of recognizing MHC—I molecules and capable of killing MHC—I positive or negative cells when produced in vivo.

57. An isolated ITNK cell according to claim **56** obtainable, or obtained, from a T cell or pro-T cell.

58. An isolated ITNK cell obtained by carrying out a process as defined in claim **46**.

59. An isolated target T cell or target pro-T cell including at least one Bcl11b gene product and/or protein product the activity and/or effect of which has been modulated compared to the corresponding gene and/or protein product in a precursor T cell or precursor pro-T cell, so that the target T cell or target pro-T cell is capable of converting to an ITNK cell.

60. An isolated target T cell or pro-T cell obtained by carrying out a process as defined in claim **47**.

61. A method of treating a human or non-human mammal subject suffering from, or susceptible to disease such as cancer or viral infection, comprising administering to said subject a therapeutically effective amount of ITNK cells according to claims **54-56**.

62. A method of treating a human or non-human mammal subject suffering from, or susceptible to disease such as cancer or viral infection, comprising administering to said subject a therapeutically effective amount of cells according to claim **57** or **58**.

63. The method of claim **50** or **51**, wherein said deletion comprises at least part of exon 4 of said Bcl11b gene.

64. The isolated ITNK cell of claim **56**, wherein said cell is characterized by a gene expression profile more similar to that of LAK cells than the parental cells from which they were developed.

65. The isolated ITNK cell of claim **56**, wherein said one or more specific NK genes are selected from the group consisting of ZFP105, IL2Rβ3, Id2, JAK1, NKG2D, NKG2A/C/E, B220, Rog (Zbtb32), Tnfrsfθ, Cdknic, Trail, Perforin, Interferonˆ, NK1.1, NKp46, E4 bp4, NKG7, KLRD1, LTA, PLCG2, Ly49C/1 and Ly49G2.

66. The isolated ITNK cell of claim **56**, wherein said one or more T lineage genes are selected from the group consisting of Notchi, Est1, Hes1, Gata3, Deltaxi, TCRβ, CD3, TcM 1 IL7R, T-bet and/or CD8a.

67. The isolated ITNK cell of claim **56**, wherein said cell killing ability is characterized by the ability to prevent or ameliorate tumour formation or growth, the ability to kill stromal cells, tumour cells, or infected cells, in comparison to the precursor cell used (parent T cells or pro T cells).

* * * * *